

# Discovery of Novel Traits in Seed-Propagated *Lilium*: Non-vernalization-requiring, Day-neutral, Reflowering, Frost-tolerant, Winter-hardy *L. xformolongi*. II. Photoperiodism in Parents and Hybrids

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## ABSTRACT

The discovery of seed-propagated lily hybrids which flower in <1 year from sowing in any photoperiod presents unique opportunities for transforming lilies. Recent research documented such hybrids to possess additional traits such as reflowering capabilities, frost tolerance, and winter hardiness. Objectives of this research were to examine *Lilium xformolongi* hybrids, backcrosses, and parental species (*L. formosanum*, *L. longiflorum*) in photoperiods (SD/LD) to clarify flowering in more stringent environments (growth chambers, greenhouses) than previously. Case cooled (CC) and non-CC bulbs (*L. formosanum*, *L. longiflorum* 'Nellie White'); non-vernalized *L. xformolongi* seed-propagated cultivars (5 cultivars, 9 seed lots), *L. longiflorum* 'Snow Trumpet', and *L. xformolongi* backcrosses (5 BC<sub>1</sub>F<sub>1</sub>) were tested. Seed germination ranged from 4% to 83.3%; yield potential was similar. Flowering *L. xformolongi* (growth chamber) did not differ from *L. longiflorum* 'Nellie White' for VBD. Cultivar x photoperiod interactions were not significant except flowering date ( $P = 0.04$ ). 'Nellie White' (CC) flowered in 213 d, while *L. xformolongi* cultivars flowered in 247 d ('Sakigake Raizan') to 306 d ('Raizan No. 3') from sowing. Non-vernalized *L. formosanum*, *L. longiflorum* bulbs never flowered in either photoperiod or environment. Both VBD and flowering date were highly heritable and correlated. Regardless of photoperiod and environment, seed-propagated *L. xformolongi* flowered in <1 year. One backcross was day-neutral for flowering. Two *L. xformolongi* BC<sub>1</sub>F<sub>1</sub> and *L. longiflorum* 'Snow Trumpet' produced significantly less leaves than 'Nellie White'. Leaf number ( $h^2 = 0.83$ ) was not as tightly linked in *L. xformolongi* as 'Nellie White'. Plant height in *L. formosanum* (CC bulbs, several backcross *L. xformolongi* hybrids) did not differ from 'Nellie White'. In contrast, only 'Sakigake Raizan' was taller than 'Nellie White' in growth chambers.

**Keywords:** day neutrality, flowering, heritability (broad-sense), leaf number, *Lilium formosanum*, *Lilium longiflorum*, photoperiodism, visible bud date

**Abbreviations:** CC, case-cooled, LD, long days, SD, short days, VBD, visible bud date

## INTRODUCTION

Commercial floriculture has many crop anomalies. For instance, N. American Easter lily is the only major flowering potted crop not propagated by one or more central propagators (Zlesak and Anderson 2003). Rather, each lily grower propagates their own clone of 'Nellie White'. The ability to respond to projected increased demand (Prince and Prince 2003) may be limited and leads to widening of the clonal genetic variation (Anderson *et al.* 2010a). Equally challenging are propagator and grower 'traditions', *e.g.* vegetative propagation of bulb crops with cultivar-specific production recipes, *e.g.* for *Lilium longiflorum* Thunb. 'Nellie White' (Dole and Wilkins 2005). Challenges of directing change in existing crops often require creative and strategic breeding objectives.

Commercial potted and cut lilies in the U.S. are 100% vegetatively propagated (bulbs, bulb divisions, aerial axillary stem bulbils), rather than sexually (seed) (Dole and Wilkins 2005). Garden cultivars are predominantly vegetatively propagated (McRae 1998). Oddly enough, however, white trumpet cut lilies in Japan and Korea (*L. xformolongi*) are seed-propagated (Dai-Ichi Seed Co. 1999), with ~15 million stems/year produced in Japan (Okazaki 1996). The total planted area for all cut lilies in Japan for 2011 is 165 ha, producing 17.2 million stems (U.S. Dept. of Agriculture, Foreign Agricultural Service 2011). Commercial production follows that of vegetatively-propagated cut lily bulbs, namely a one-time harvest and seedling disposal. These

complex hybrids of *L. xformolongi* are derived from crossing *L. formosanum* Wallace x *L. longiflorum*, both closely related white trumpet lilies (Section *Leucolirion*; Okazaki 1996), followed by subsequent inbreeding and backcrossing (Anderson and Dunn 2003). In the Ryuku Islands (27° N lat.), wild *L. longiflorum* bulbs (2-3 year old) flower after long days (LD) and a short duration of < 21°C (Wilkins 1973), whereas *L. formosanum* populations segregate for flowering from seed in 1-2 year (Shii 1983).

Dole and Wilkins (1996) identified the need for day neutral, non-vernalization-requiring Easter lilies. Previous research (Anderson 2003; Anderson *et al.* 2010b) characterized *L. xformolongi* hybrid, commercial cultivars to determine whether these and other traits existed in greenhouse and field environments. Non-cooled commercial hybrids of *L. xformolongi* 'Augusta F<sub>1</sub>', 'Raizan No. 1', 'Raizan No. 2', 'Raizan No. 3', and 'Sakigake Raizan' completed juvenility and commenced stem elongation/floral initiation soon after transplanting, regardless of short (SD) or long day (LD) photoperiods (Anderson *et al.* 2010b). On average, comparison *L. longiflorum* 'Nellie White' (vernalized bulbs) flowered in 213 d, while *L. xformolongi* cultivars flowered in 237 d ('Sakigake Raizan', 'Raizan No. 1' – LD) to 306 d ('Raizan No. 3') from sowing with high flower bud counts (7/plant in 'Augusta F<sub>1</sub>'). In previous studies, flowering took 9 months (~270 d) with a vernalization treatment (Mynett 1997). Photoperiod did not have a significant effect on leaf unfolding rates, plant height, leaf number, or flowering dates (Anderson *et al.* 2010). Many,

but not all of the cut flower hybrids were taller than 'Nellie White'. Most traits were highly heritable and all *L. xformolongi* flowered in <1 year due to a short juvenility period (Anderson *et al.* 2010b).

Field experiments (45°N lat.) resulted in the discovery of additional traits in the *L. xformolongi* hybrids. Since vernalization (cold) was not required for flowering, shoots were continually initiated throughout the growing season (Anderson *et al.* 2010b). 'Sakigake Raizan' averaged as many as four shoots/plant, while 'Nellie White' produced only one; several selections continuously flowered in the greenhouse for >2 years. *L. xformolongi* reflowered continuously during the growing season (Anderson *et al.* 2010b), which was unexpected since this trait had never been observed (Mynett 1997; Rhee *et al.* 2005; Roh and Sim 1996; Watanabe 1993). This allowed for the first-ever frost-tolerance screening, which ranged from 25% ('Sakigake Raizan') to 75% ('Augusta F<sub>1</sub>') (Anderson *et al.* 2010b). After several frosts, numerous hybrids remained in full flower until a killing freeze. Winter survival was also recorded and varied from 0% ('Augusta F<sub>1</sub>', 'Raizan No. 3') to 87.5% ('Sakigake Raizan') in *L. xformolongi*.

After the initial characterization of these unique traits in seed-propagated *L. xformolongi*, experimentation commenced to elucidate responses in test environments. For instance, Anderson and Dunn (2003) discovered that many hybrids were self-compatible which allowed for the creation of inbreds. Inbreeding depression was evident across multiple inbred lines, although highly fertile inbred parents could be derived for subsequent backcrossing to both parents and to create dwarf hybrids (Anderson and Dunn 2003). Zlesak and Anderson (2010) reported that the lack of a cold (vernalization) requirement in *L. xformolongi* derived from the *L. formosanum* parent with the trait being controlled by two genes, *VER1* and *VER2*. The objectives of this research were to examine *L. xformolongi* hybrids, backcrosses, and parental species for post-emergence photoperiodic requirements (flower bud initiation/development), morphological / reproductive traits, and determine heritability.

## MATERIALS AND METHODS

### Germplasm

Seeds of *L. xformolongi* F<sub>1</sub> hybrids included some of the seed lots used previously (Anderson *et al.* 2012) as well as newer ones: 'Raizan No. 1' (MAFF Reg. #2835; Seed Lot Nos. 8241; 8159), 'Raizan No. 2' (Seed Lot Nos. 8086; 8148), 'Raizan No. 3' (MAFF Reg. #2836; Seed Lot Nos. 8418, 8154), and 'Sakigake Raizan' (= 'Raizan Herald'; Seed Lot Nos. 2029; 9097) were obtained from Dai-Ichi Seed Co., Ltd. (Tokyo, Japan—now owned by Murakami Seed Co., Ltd.) and seeds of 'White Lancer' (Seed Lot No. 1210) from Park Seed Co. (Greenwood, SC, USA), in 1999-2000. Several seed lots were the same as those used in the first experiments to characterize the identified traits (Anderson *et al.* 2010b). Seeds of *L. longiflorum* 'Snow Trumpet' (Seed Lot No. 1182) were purchased from Sakata Seed Corp. (Yokohama, Japan). Seeds of wild *L. formosanum* were obtained from Mary Queitzsch (Seed Lot No. 502, half-sib family, Madison Mills, Virginia, U.S.A.).

Similar to Anderson *et al.* (2011), bulbs of *L. longiflorum* 'Nellie White' (22.9-25.4 cm or 9-10" circumference) were donated by Ollie Hoffman, Fred Gloeckner Co. (Harrison, New York, U.S.A.) after fall digging in October 2000. *L. formosanum* bulbs (12.7-15.2 cm or 5-6" circumference) were purchased from

**Table 2** Percent germination and % yield potential of seed-propagated *Lilium formosanum*, *L. longiflorum* species and *L. xformolongi* hybrid and backcross (BC<sub>1</sub>F<sub>1</sub>) cultivars.

Cultivar or hybrid	Seed Lot No.	No. seeds sown (N)	Percent germination	Percent yield potential
<i>L. formosanum</i>	502	288	41.0%	39.5%
<i>L. longiflorum</i>				
Snow Trumpet	1182	96	77.1	73.5
<i>L. xformolongi</i>				
Raizan No. 1	8159	96	66.7	28.0
	8241	96	72.9	72.0
Raizan No. 2	8148	96	34.0	32.0
	8086	96	64.6	63.8
Raizan No. 3	8418	96	28.0	28.0
	8154	96	67.8	65.0
Sakigake Raizan	9097	96	26.0	18.0
	2029	96	18.8	18.8
White Lancer	1210	96	79.2	75.0
<b>Hybrid BC<sub>1</sub>F<sub>1</sub></b>				
00L-13	---	96	69.8	65.0
00L-74	---	96	77.1	75.0
00L-96	---	96	37.5	37.0
00L-116	---	96	83.3	80.0
00L-136	---	96	43.8	40.0

Heronswood Nursery (Kingston, Washington, U.S.A.).

Interspecific backcross hybrids were made by the University of Minnesota Herbaceous Perennial Breeding Program using hybrid and inbred parents (Anderson and Dunn 2003). Five BC<sub>1</sub>F<sub>1</sub> progeny (containing two species dosages of *L. longiflorum* and one species dose of *L. formosanum* or *vice versa*) were created for use in this study (Table 1).

### Photoperiod Experiment – Greenhouse and Growth Chambers.

In this experiment, both greenhouses and growth chambers were used as test environments, coupled with photoperiod (SD, LD in both environments) to determine photoperiod response; parents and interspecific hybrid seedlings (n=19 accessions, due to growth chamber space constraints) were studied. *Lilium longiflorum* 'Nellie White' (case cooled or CC and non-vernalized) 54-60 cm bulbs and 'Snow Trumpet' seeds (Lot No. 1182), *L. formosanum* bulbs (CC) and seeds (Lot. No. 502) were included in this experiment. Vernalized and non-vernalized bulbs and non-vernalized *L. longiflorum* seedlings served as controls. For comparative purposes, three *L. xformolongi* seed lots (Dai-Ichi Lot Nos. 8159, 8148, 9097) were the same as those used in previous screening experiments (Anderson *et al.* 2010b). With the exception of *L. formosanum* (n=288 seeds), n=96 seeds/lot were sown for all seed-propagated accessions in this experiment (Table 2). There were n = 5 replicates/treatment/accession for a total of N=380 experimental units.

The experiment commenced when the lily bulbs were received on 24 Oct 2000. On that date, cooling of CC bulbs began and all seeds were sown. CC bulbs were cooled at 4.4°C in moist moss peat for 6 weeks (1,000 h) (Stuart 1954). Non-vernalized lily bulbs were stored at 21°C in moist moss peat in darkness for 4 weeks and then potted into 24 cm Jumbo Junior pots using Universal soil medium (SB300, SunGro Horticulture, Bellevue, WA, USA) and rooted in the same conditions for 2 weeks. Seed-propagated *L. xformolongi* hybrids, F<sub>1</sub>BC<sub>1</sub>, and parental cultivars were germinated using the same environmental conditions and protocols as Anderson *et al.* (2010b) under LD before being randomized to environments and photoperiods. At the termination of the vernalization for CC bulbs, non-rosetted seedlings were transplanted in

**Table 1** Hybrid code of *Lilium* backcross F<sub>1</sub> (BC<sub>1</sub>F<sub>1</sub>) progeny and their parental species used for comparative photoperiodic responses.

BC <sub>1</sub> F <sub>1</sub> hybrid code	Female parent(s)	x	Male parent(s)
00L-13	[( <i>L. formosanum</i> x <i>L. longiflorum</i> ) A-2]	X	<i>L. longiflorum</i> 'Nellie White'
00L-74	[( <i>L. formosanum</i> x <i>L. longiflorum</i> ) R-27]	X	<i>L. longiflorum</i> 'Nellie White'
00L-96	[( <i>L. formosanum</i> x <i>L. longiflorum</i> ) R-34]	X	<i>L. longiflorum</i> 'Nellie White'
00L-116	[( <i>L. formosanum</i> x <i>L. longiflorum</i> ) R-6]	X	<i>L. longiflorum</i> 'Nellie White'
00L-136	<i>L. longiflorum</i> 'Nellie White'	X	[( <i>L. formosanum</i> x <i>L. longiflorum</i> ) R-1]

**Table 3** F-tests and significance (\*, \*\*, \*\*\*, ns =  $P \leq 0.05$ ,  $P \leq 0.01$ ,  $P \leq 0.001$ , not significant, respectively) from Analyses of Variance (ANOVA) for number of days to visible bud date (VBD), number of days to first flower, plant height (cm), inflorescence length (cm), number of leaves, number of flowers, and number of shoots/bulb of *Lilium* accessions (entire grouping of species and hybrids) in environments (greenhouse, growth chambers) and photoperiods (short vs. long days).

Factor	Visible bud date <sup>a</sup>	Leaf unfolding rate/wk <sup>a</sup>	Plant height (cm) <sup>a</sup>	Total leaf no. <sup>a</sup>	Flower bud count <sup>a</sup>	No. days to flowering <sup>b</sup>	No. shoots / bulb <sup>b</sup>
Genotype (Accessions)	10.7***	13.3***	15.3***	3.6***	35.0***		
Environment	7.1***	2.3***	509.3***	73.1***	119.6***		
Photoperiod	60.7***	49.6***	0.5ns	2.5ns	3.8*		
Genotype x Environment	8.4***	8.9***	3.9***	3.3**	3.6***		
Genotype x Photoperiod	2.6**	1.3ns	1.4ns	2.4**	1.7ns		
Environment x Photoperiod	23.9***	14.5***	1.9ns	0.03ns	2.9ns		
Genotype x Environment x Photoperiod	1.7ns	0.4ns	1.5ns	2.1ns	1.5ns		

\*, \*\*, \*\*\*, ns denote significant  $P$ -values at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , and not significant, respectively

<sup>b</sup>ANOVA tests are not valid for these traits as the ANOVA assumptions are violated and no transformation could make the variances equal. Instead, refer to the non-parametrical statistical tests performed for these two parameters (see text)

24 cm Jumbo Junior pots using Universal soil medium (SB300, SunGro Horticulture, Bellevue, WA, USA); all seedlings, CC and non-vernalized bulbs were immediately placed into the environments and photoperiods on 13 Dec 2000. Within each photoperiod treatment, the plants were spaced 24 cm O.C. in a CRD design. For 'Raizan No. 3' and 'White Lancer' seed lots, due to insufficient number of germinated seedlings at transplanting, seedlings were grown only in the chamber environment. Data from these seed lots were omitted from the statistical analyses.

Both the LD and SD environments in the growth chambers had 8 h of photosynthetically active radiation (PAR) with light provided by a combination of incandescent and fluorescent lamps ( $200 \mu\text{mol s}^{-1} \text{m}^{-2}$ ). Long days were provided by an additional 4 h night interruption (22:00 pm – 02:00 am) using incandescent light ( $2 \mu\text{mol s}^{-1} \text{m}^{-2}$ ) (Anderson and Ascher 2004). The greenhouse ( $45^\circ\text{N}$  lat., St. Paul, MN USA) photoperiod treatments received the same duration of light; the lighting during 08:00 am – 16:00 pm was natural light was supplemented with 400 watt sodium vapor high intensity discharge (HID) lamps providing supplemental light ( $\sim 200 \mu\text{mol s}^{-1} \text{m}^{-2}$ ). The LD environment received an additional 4-h night interruption (22:00 pm – 02:00 am) using incandescent light ( $2 \mu\text{mol s}^{-1} \text{m}^{-2}$ ). Both greenhouse and growth chambers were set at  $21^\circ\text{C}$  constant day/night temperatures, although the ability for strict temperature control in the greenhouse was suboptimal. Day/night temperatures in the greenhouse were  $26.1 \pm 4.4^\circ\text{C}/18.3 \pm 1.2^\circ\text{C}$  day/night (SD),  $26.7 \pm 4.4^\circ\text{C}/18.9 \pm 1.1^\circ\text{C}$  (LD) and the growth chambers were  $21.1 \pm 0.1^\circ\text{C}$  (SD) and  $21.4 \pm 0.8^\circ\text{C}$  (LD). Plants were grown under these photoperiods for 7 months, terminating on 13 July 2001. After the first stem flowered and data were recorded, plants in greenhouse environment (SD, LD photoperiods) were cut back and repotted into 36 cm standard pots for subsequent shoot regrowth and flowering evaluation in the same photoperiods and greenhouse environment for five additional months (terminating 13 Dec 2001).

The following data were collected on all plants: seed germination (weekly counts), % germination, % yield potential, number of days to VBD, leaf unfolding rate/wk, plant height from the soil surface (cm), number of leaves on the first (main) stem, inflorescence length (cm); measured from the last leaf to the top of the inflorescence), flower bud count, number of days to flowering, and the number of shoots/bulb. Data were analyzed using SPSS (University of Chicago, Chicago, IL, USA). Analyses of Variance were performed. Mean separations within each environment, with photoperiods pooled (since there were  $< 3$  groups), were determined using the Tukey's Honestly Significant Difference (HSD) test at  $\alpha = 0.05$ . Non-parametric statistical tests (Kruskal-Wallis X2) for flower bud counts and number of shoots/bulb were grouped by environments (greenhouse vs. chamber) and photoperiods (SD vs. LD) to test for significance. Broad sense heritability ( $h^2$ ) estimates (entry-mean basis) were calculated (Anderson *et al.* 2010b) within each environment for leaf unfolding rates, plant height, inflorescence length, number of leaves, number of flower buds, VBD, flowering date, and the number of shoots/bulb.

## RESULTS

Percent germination ranged from 18.8% ('Sakigake Raizan', Seed Lot No. 2029) to 83.3% (BC<sub>1</sub>F<sub>1</sub> 00L-116) in this experiment (Table 2). Only three commercial hybrids had  $>70\%$  germination (*L. longiflorum* 'Snow Trumpet', *L. xformolongi* 'Raizan No. 1' [Seed lot No. 8241], and 'White Lancer'); the remaining commercial hybrids had lower % germination. The BC<sub>1</sub>F<sub>1</sub> hybrid seed germination range was similar to the commercial cultivars (Table 2). Overall, seed germination was lower than commercial standards (PanAmerican Seed Co. 2005).

Similar to % germination, % yield potential ranged from 18.8% ('Sakigake Raizan', Seed Lot No. 2029) to 80% (BC<sub>1</sub>F<sub>1</sub> 00L-116) (Table 2). The BC<sub>1</sub>F<sub>1</sub> hybrids yield potentials ranged from 37% (00L-96) to 80% (00L-116). In most hybrids, the yield potential is below the recommended range for commercial seed products (PanAmerican Seed Co., 2005). *L. formosanum* seedlings (Lot. No. 502), derived from wild populations had many rosetted seedlings. Rosetted seedlings also occurred in BC<sub>1</sub>F<sub>1</sub> hybrids BC<sub>1</sub>F<sub>1</sub> 00L-74 and 00L-96 (data not shown).

Genotypes and environments (greenhouse vs. growth chamber) were highly significant for all traits examined (Table 3). Likewise, photoperiod (SD vs. LD) was highly significant for the number of days to VBD and first flower, whereas leaf number was less significant (Table 3). Photoperiod had no significant effect on plant height and inflorescence length. Three-way interactions (genotype  $\times$  environment  $\times$  photoperiod) were not significant (Table 3), although two-way interactions varied in significance. For instance, all traits were significant to varying degrees for genotype  $\times$  environment interactions (Table 3). Genotype  $\times$  photoperiod interactions were significant ( $P < 0.01$ ) only for the number of days to VBD and inflorescence length. Environment  $\times$  photoperiod interactions were highly significant for the number of days to VBD and first flower (Table 3). Interestingly, all two-way interactions were significant for only one trait, the number of days to VBD. Due to the varied levels of significance in the interactions, data were not pooled.

Using the non-parametric Kruskal-Wallis Chi-square ( $\chi^2$ ) test (grouped by environment), the number of flowers were not significantly different between environments for *L. longiflorum* 'Nellie White' (CC bulbs), *L. formosanum* (CC bulbs), *L. xformolongi* 'Raizan No. 1' (Lot #8241), 'Raizan No. 2' (both seed lots), and 'Sakigake Raizan' (both seed lots) (data not shown). The number of shoots/bulb was significant for only *L. xformolongi* BC<sub>1</sub>F<sub>1</sub> 00L-74. When the data was grouped by photoperiod, Kruskal-Wallis  $\chi^2$  tests indicated that the number of flowers were significantly different for *L. longiflorum* 'Nellie White' (CC bulbs) and BC<sub>1</sub>F<sub>1</sub> 00L-96. The number of shoots/bulb was not significant for any of the tested genotypes when grouped by photoperiod.

As expected, *L. longiflorum* 'Nellie White' non-cooled bulbs did not reach VBD nor flower in either environment

or photoperiod during the experiment (**Table 4**; Lin and Wilkins 1973). It is unknown whether non-vernalized *L. formosanum* bulbs would react the same as 'Nellie White' since insufficient quantities of bulbs were available. In the greenhouse environment, CC 'Nellie White' reached VBD earlier under LD (135 d) than SD (157 d; **Table 4**); the same was true with flowering (167 d vs. 180 d, respectively). Thus, provided a vernalization (cold) treatment has been applied (Lin and Wilkins 1973), 'Nellie White' is a quantitative LD plant for flower bud initiation, as previously reported (Weiler and Langhans 1968, 1972). 'Nellie White' CC bulbs reached VBD significantly earlier than all other cultivars (**Table 4**), whereas *L. formosanum* CC bulbs were the latest. *L. longiflorum* 'Snow Trumpet', *L. formosanum* seedlings, *L. xformolongi* 'Raizan No. 2' (Lot#8086), 'Sakigake Raizan' (Lot#2029), BC<sub>1</sub>F<sub>1</sub> 00L-13, 00L-74,

00L-96, and 00L-136 all overlapped with *L. formosanum* CC bulbs in the greenhouse (**Table 4**). In the growth chamber with less day/night temperature variation, CC 'Nellie White' reach VBD (158 d) and flowered (187 d) only under LD and did so in a longer timeframe than in the greenhouse (**Table 4**). Of those cultivars flowering in the growth chamber, no genotypes differed significantly from 'Nellie White' (CC bulbs) for VBD. Other genotypes that did not reach VBD in the growth chamber environment included *L. longiflorum* 'Snow Trumpet', *L. formosanum* seedlings, and all five BC<sub>1</sub>F<sub>1</sub> hybrids (**Table 4**). *L. formosanum* (CC bulbs) did not flower under LD in either environment. *Lilium xformolongi* 'Raizan No. 1' (Lot #8241) and 'Raizan No. 2' (Lot #8086) did not flower under SD in the growth chamber only. 'Raizan No. 1' (Lot #8159) also did not flower under LD in the growth chamber (**Table 4**). Interestingly, both

**Table 4** Mean number of days to visible bud date (VBD), number of days to first flower, plant height (cm), inflorescence length (cm), number of leaves, number of flowers, and number of shoots/bulb for *Lilium longiflorum* 'Nellie White', 'Snow Trumpet', *L. formosanum*, and their interspecific *L. xformolongi* F<sub>1</sub> and BC<sub>1</sub>F<sub>1</sub> hybrids grown in two environments [greenhouse (G), growth chamber (C)] under short (SD) and long (LD) day photoperiods during 12/13/2000-7/13/2001. Mean separations (5% Tukey's HSD) in columns are for each environment, pooled for photoperiod.

<i>Lilium species</i>	Cultivar or backcross hybrid	Environments	No. days to VBD		No. days to first flower <sup>d</sup>		Plant height (cm)		Inflorescence length (cm)		
			SD	LD	SD	LD	SD	LD	SD	LD	
<i>L. longiflorum</i>	'Nellie White', case cooled bulbs	G	157 d	135	180	167	29 a	33	17 d	16	
		C	--	158 ab	--	187	12 abcde	22	-- a	7	
	'Nellie White', non-cooled bulbs	G	--	--	--	--	29 abc	62	--	--	
		C	--	--	--	--	8 abcd	18	--	--	
	'Snow Trumpet', Lot #1182	G	206 abc	214	238	231	76 abcd	34	14 abcd	13	
		C	--	--	--	--	10 abc	8	--	--	
<i>L. formosanum</i>	Case cooled bulbs	G	233 a	240	260	--	68 abc	53	9 abc	12	
		C	124 a	122	143	--	15 cdef	29	3 b	16	
	Seedlings, Lot #502	G	258 ab	222	262	250	68 cdefg	83	8 a	9	
		C	--	--	--	--	10 abc	7	--	--	
	<i>L. xformolongi</i>	'Raizan No. 1', Lot #8241	G	211 c	196	244	227	101 fg	98	10 abc	10
			C	--	208	29	30	8 def	8	60 a	47
F <sub>1</sub> hybrids	'Raizan No. 1', Lot #8159	G	222 c	223	93	99	14 efg	15	60 abcd	57	
		C	240 ab	152	257	-- <sup>a</sup>	41 def	21	6 a	1 <sup>a</sup>	
	'Raizan No. 2', Lot #8086	G	228 abc	200	254	229	100 fg	100	6 abc	14	
		C	194 ab	127	--	217	22 bcdef	27	-- <sup>a</sup>	6	
	'Raizan No. 2', Lot #8148	G	196 c	191	226	220	100 g	109	14 bcd	16	
		C	228 ab	161	262	204	49 ef	19	8 a	3	
	'Raizan No. 3', Lot #8418	G <sup>b</sup>	219	--	244	--	12	8	-- <sup>a</sup>	--	
		C	--	--	--	--	20	6	--	--	
	'Raizan No. 3', Lot #8154	G <sup>b</sup>	220	199	244	228	88	82	11	11	
		C	234 b	172	259	188	41 ef	18	6 a	6	
	'Sakigake Raizan', Lot #9097	G	201 bc	209	230	240	107 g	109	16 cd	13	
		C	234 b	212	259	237	44 f	38	3 a	6	
'White Lancer', Lot #1210	G <sup>b</sup>	220	199	244	228	88	82	11	11		
	C	--	--	--	--	23	8	--	--		

**Table 4** (Cont.)

<i>Lilium species</i>	Cultivar or backcross hybrid	Environ ments	No. days to VBD		No. days to first flower <sup>d</sup>		Plant height (cm)		Inflorescence length (cm)	
			SD	LD	SD	LD	SD	LD	SD	LD
BC <sub>1</sub> F <sub>1</sub> hybrids	00L-13	G	220 abc	220	253	238	68 abcd	47	12 abcd	15
		C	--	--	--	--	8 abc	9	--	--
	00L-74	G	222 abc	215	250	237	56 abcdef	74	13 abcd	11
		C	--	--	--	--	7 a	8	--	--
	00L-96	G	256 ab	218	266	243	57 abcdef	72	7 ab	10
		C	--	--	--	--	10 ab	7	--	--
	00L-116	G	214 c	195	240	223	14 bcdefg	16	51 abcd	45
		C	--	--	--	--	9 abc	8	--	--
	00L-136	G	274 abc	203	277	233	32 ab	40	-- <sup>z</sup> abcd	12
		C	--	--	--	--	9 a	6	--	--

seed-propagated parental species (*L. longiflorum* ‘Snow Trumpet’, *L. formosanum* Lot#502) reached VBD and flowered in the greenhouse (both photoperiods). ‘Snow Trumpet’ was a facultative SD plant for flower bud initiation and early development (no. of days to VBD) but a facultative LD plant for later flower development (no. days to first flower) in the greenhouse. In contrast, but in the same environment, *L. formosanum* Lot#502 was a facultative LD plant for both flower bud initiation and development. ‘Snow Trumpet’ reached VBD and flowered several days or weeks earlier than *L. formosanum* seedlings in the greenhouse environment (Table 4); neither reached VBD nor flowered in the growth chamber. Visible bud date was very highly and significantly correlated with flowering date in both the greenhouse and growth chamber environments ( $r = 0.96$ ; Table 5). Broad sense heritability for VBD was higher in the greenhouse environment was  $h^2 = 0.49$  with a 95% C.I. of 0.39 to 0.59 than in the growth chamber  $h^2 = 0.16$  with a 95% C.I. of 0.13 to 0.19. Flowering date had similar broad sense heritability values:  $h^2 = 0.39$  (95% C.I. 0.26-0.52) in the greenhouse and  $h^2 = 0.29$  (95% C.I. 0.24-0.34) in the growth chamber.

Regardless of photoperiod and environment, when seed-propagated *L. longiflorum*, *L. formosanum*, and *L. xformolongi* reached VBD and flowered they did so in <1 year (< 365 d) from sowing (Table 4). A general trend with *L. xformolongi* hybrids was for earlier VBD and flowering under LD than SD regardless of the environment (Table 4). In a few cultivars, this trend was reversed in one of the environments, e.g. ‘Raizan No. 1’ (Lot#8159) reached VBD 5d earlier under SD than LD in the greenhouse, whereas seedlings were at VBD 88d later in SD in the growth chamber (Table 4). In one hybrid, BC<sub>1</sub>F<sub>1</sub> 00L-13, mean VBD was identical in both SD and LD (200 d; Table 4) in the greenhouse environment; thus, 00L-13 is day-neutral for flower bud initiation (Erwin and Warner 2002; Anderson and Ascher 2004). Other hybrids flowered in both greenhouse photoperiods but differed in leaf numbers (SD = 51, LD = 45, Table 4), e.g. BC<sub>1</sub>F<sub>1</sub> 00L-116 (Fig. 1). *Lilium formosanum* CC bulbs flowered faster in the growth chamber under SD (143 d) than LD (260 d). The BC<sub>1</sub>F<sub>1</sub> hybrids neither reached VBD nor flowered in the growth chamber, regardless of photoperiod. This was identical to the responses found for *L. longiflorum* ‘Snow Trumpet’ and *L. formosanum* Lot#502 in the same environment and photoperiod treatments.

Plant height varied widely across photoperiods and environments and was dependent on whether or not flower bud initiation had occurred (Table 4). If flower bud initiation had not occurred, leaf making continued (De Hertogh

1996). Likewise, in the growth chamber and greenhouse environments, plant height was significantly correlated with VBD ( $r = 0.64$ ,  $r = 0.28$ , Table 5) and flowering date ( $r = 0.62$ ,  $r = 0.41$ ), respectively. Most often, when plants were still in the juvenile phase (vegetative) and had not undergone flower bud initiation and early development (VBD), the genotypes remained either rosetted (short plant height of 6-18 cm in the growth chamber for both photoperiods – ‘Nellie White’ non-cooled, ‘Snow Trumpet’, *L. formosanum* Lot#502, BC<sub>1</sub>F<sub>1</sub> 00L-13, 00L-74, 00L-96, 00L-116, 00L-136; Table 3) or elongated to comparable heights (29-62 cm in the greenhouse, both photoperiods – ‘Nellie White’) for those reaching VBD. Broad sense heritability for height was similar between the greenhouse ( $h^2 = 0.88$ , 95% C.I. = 0.80-0.96) and growth chamber ( $h^2 = 0.78$ , 95% C.I. = 0.67-0.89). Flowering clonal ‘Nellie White’ (CC bulbs) was shorter in the growth chamber under LD (22 cm), compared with the greenhouse environment (33 cm, Table 4), although statistical comparisons were not possible. In the greenhouse, mean separations of plant height of the *L. formosanum* CC bulbs, BC<sub>1</sub>F<sub>1</sub> 00L-136, 00L-96, 00L-74, and 00L-13 all overlapped with ‘Nellie White’ CC bulbs, whereas *L. formosanum* seedlings (68-83 cm; seed Lot#502) and the remaining genotypes did not (Table 4). ‘Sakigake Raizan’ (Lot#9097) was significantly taller than all other genotypes in the greenhouse environment. Thus, this seed-propagated *L. xformolongi* would require plant growth regulator applications to control plant height, if it were to be grown as a potted Easter lily crop. Only ‘Sakigake Raizan’ (Lot#9097) was the only hybrid that differed significantly from ‘Nellie White’ for plant height in the growth chamber, based on mean separations (Table 4). 00L-136 and all other BC<sub>1</sub>F<sub>1</sub> hybrids did not flower in the growth chamber, unlike most of the commercial *L. xformolongi* cultivars (Table 4).

For those genotypes flowering in either photoperiod or environment, inflorescence lengths ranged from 3 cm to 17 cm, when discounting genotypes with aborted flower buds (e.g. 1 cm length for ‘Raizan No. 1’, Lot#8159, LD, growth chamber; Table 4). With the exception of *L. formosanum* (CC bulbs, LD), all genotypes flowering in both environments always had taller inflorescences in the greenhouse than the growth chamber. Inflorescence lengths in the greenhouse, regardless of photoperiod, were in a similar range of 9 cm to 17 cm for all flowering genotypes, including ‘Nellie White’ (Table 3). ‘Snow Trumpet’, ‘Raizan No. 1’ (Lot#8159), ‘Raizan No. 2’ (Lot#8148), ‘Sakigake Raizan’ (Lots #2029, 9097), BC<sub>1</sub>F<sub>1</sub> 00L-13, 00L-74, 00L-116, and 00L-136 mean inflorescence lengths did not differ significantly from ‘Nellie White’ CC bulbs (Table 4). Inflorescence length was not significantly correlated with

either VBD ( $r = -0.19$ , **Table 5**), flowering date ( $r = 0.1$ ), or plant height ( $r = -0.15$ ) in the growth chamber environment. In contrast, similar correlations in the greenhouse environment were significant: VBD ( $r = -0.51$ ), flowering date ( $r = -0.52$ ), and plant height ( $r = -0.19$ ) (**Table 5**). Broad sense heritability estimates were low in both the greenhouse ( $h^2 = 0.53$ , 95% C.I. = 0.52-0.55) and growth chamber ( $h^2 = 0.35$ , 95% C.I. = 0.28-0.42).

Leaf number for both photoperiods ranged from 22 to 192 in the greenhouse to 11-146 in the growth chambers (**Table 4**). The lowest range of leaf numbers primarily occurred in plants, which did not reach VBD or flower due to resetting (e.g. 'Snow Trumpet', growth chamber, 13-14 leaves). Likewise, in several instances, higher leaf numbers occurred in plants which also had not reached VBD or flowering but were not resetted and kept producing leaves, e.g. 'Nellie White', non-cooled bulbs in the greenhouse produced an average of 171-192 leaves (**Table 4**). Leaf numbers were significantly and negatively correlated with VBD

( $r = -0.45$ , **Table 5**) and flowering date ( $r = -0.51$ ) in the greenhouse, but positively and significantly correlated with plant height ( $r = 0.78$ ) in the growth chamber and inflorescence length ( $r = 0.19$ ) in the greenhouse (**Table 5**). Broad sense heritability for leaf number were lower in the greenhouse ( $h^2 = 0.83$ , 95% C.I. = 0.67-0.99) and growth chamber ( $h^2 = 0.93$ , 95% C.I. = 0.87-0.99).

Genotypes BC<sub>1</sub>F<sub>1</sub> 00L-136, 00L-13, and 'Snow Trumpet' produced the least number of leaves, significantly fewer leaves than 'Nellie White' CC bulbs in the greenhouse (**Table 4**). In the growth chamber, nearly all seed-propagated cultivars produced significantly less leaves than CC 'Nellie White'. The exceptions were 'Raizan No. 1' (Lot#8241), 'Raizan No. 2' (Lots#8086, 8148); increased plant height in these hybrids compared with 'Nellie White' is attributable to longer internodes on the main stem. In many cases, the photoperiod with higher leaf numbers switched between environments within each respective clonal genotype or seedling population (**Table 4**). For example,

**Table 4 (Cont.)**

<i>Lilium species</i>	Cultivar or backcross hybrid	Environments	No. leaves		Leaf unfolding rate/wk <sup>c</sup>		No. flowers <sup>c</sup>		No. shoots per bulb <sup>c</sup>		
			SD	LD	SD	LD	SD	LD	SD	LD	
<i>L. longiflorum</i>	'Nellie White', case cooled bulbs	G	111 <sup>e</sup>	98	5	5	111	98	5	5	
		C	61 <sup>gh</sup>	106	--	5	61	106	--	5	
	'Nellie White', non-cooled bulbs	G	171 <sup>f</sup>	192	--	--	171	192	--	--	
		C	56 <sup>gh</sup>	119	--	--	56	119	--	--	
	'Snow Trumpet', Lot #1182	G	45 <sup>a</sup>	22	2	1	45	22	2	1	
		C	14 <sup>ab</sup>	13	--	--	14	13	--	--	
<i>L. formosanum</i>	Case cooled bulbs	G	88 <sup>d</sup>	67	3	2	88	67	3	2	
		C	146 <sup>h</sup>	91	8	5	146	91	8	5	
	Seedlings, Lot #502	G	73 <sup>abcd</sup>	41	2	1	73	41	2	1	
		C	20 <sup>abcdef</sup>	18	--	--	20	18	--	--	
	<i>L. xformolongi</i>	'Raizan No. 1', Lot #8241	G	63 <sup>cd</sup>	59	2	2	63	59	2	2
			C	2 <sup>efgh</sup>			2	2		2	
F <sub>1</sub> hybrids	'Raizan No. 1', Lot #8159	G	2 <sup>abcd</sup>			2	2		2		
		C	34 <sup>cdefg</sup>	41	1	2	34	41	1	2	
	'Raizan No. 2', Lot #8086	G	60 <sup>bcd</sup>	61	2	2	60	61	2	2	
		C	41 <sup>efgh</sup>	52	1	3	41	52	1	3	
	'Raizan No. 2', Lot #8148	G	60 <sup>bcd</sup>	62	2	2	60	62	2	2	
		C	59 <sup>defgh</sup>	36	2	2	59	36	2	2	
	'Raizan No. 3', Lot #8418	G <sup>b</sup>									
		C	23	19	<1	--	23	19	<1	--	
	'Raizan No. 3', Lot #8154	G <sup>b</sup>									
		C	49	12	--	--	49	12	--	--	
	'Sakigake Raizan', Lot #2029	G	59 <sup>abc</sup>	45	2	2	59	45	2	2	
		C	30 <sup>bcdefg</sup>	39	1	2	30	39	1	2	
'Sakigake Raizan', Lot #9097	G	57 <sup>bcd</sup>	65	2	2	57	65	2	2		
	C	65 <sup>cdefg</sup>	36	2	1	65	36	2	1		
'White Lancer', Lot #1210	G <sup>b</sup>										
	C	32	14	--	--	32	14	--	--		

**Table 4** (Cont.)

<i>Lilium species</i>	Cultivar or backcross hybrid	Environ ments	No. leaves		Leaf unfolding rate/wk <sup>c</sup>		No. flowers <sup>c</sup>		No. shoots per bulb <sup>c</sup>	
			SD	LD	SD	LD	SD	LD	SD	LD
BC <sub>1</sub> F <sub>1</sub> hybrids	00L-13	G	45 a	30	1	1	45	30	1	1
		C	18 abcd	15	--	--	18	15	--	--
	00L-74	G	42 ab	36	1	1	42	36	1	1
		C	11 a	9	--	--	11	9	--	--
	00L-96	G	51 abc	40	1	1	51	40	1	1
		C	20 abc	17	--	--	20	17	--	--
	00L-116	G	2 abc			2	2			2
		C	15 abc	14	--	--	15	14	--	--
	00L-136	G	36 a	33	1	1	36	33	1	1
		C	13 ab	13	--	--	13	13	--	--

<sup>a</sup>Flower buds aborted after visible bud date and the floral scape did not elongate.

<sup>b</sup>Insufficient number of germinated seedlings to include in both environments. Thus, seedlings were only grown in the chamber environment. Data from these seed lots were omitted from the statistical analyses.

<sup>c</sup>ANOVA tests are not valid for these traits as the ANOVA assumptions are violated and no transformation could make the variances equal.

<sup>d</sup>Insufficient data in the growth chamber environment to perform mean separations.

**Table 5** Correlation (r values) matrix and significance (P values a) between visible bud date, flowering date, plant height, inflorescence length, total leaf number, flower bud count, and no. of shoots/bulb for greenhouse and growth chamber environments.

Factor	Visible bud date	Leaf unfolding rate / wk	Plant height (cm)	Total leaf no.	Flower bud count	No. days to flowering	No. shoots / bulb
Greenhouse							
Visible bud date	----						
Flowering date	0.95 ***	----					
Plant height	0.28 **	0.41 **	----				
Inflorescence length	-0.51 **	-0.52 **	-0.19 *	----			
Total leaf number	-0.45 **	-0.51 **	-0.52 ns	0.19 *	----		
Flower bud count	-0.49 **	-0.54 **	-0.07 ns	0.18 ns	0.77 **	----	
No. shoots/bulb	0.01 ns	0.05 ns	0.05 ns	-0.03 ns	-0.16 *	-0.23 **	----
Growth Chamber							
Visible bud date	----						
Flowering date	-0.95 **	----					
Plant height	0.64 **	0.62 **	----				
Inflorescence length	-0.19 ns	0.10 ns	-0.15 ns	----			
Total leaf number	-0.03 ns	-0.26 ns	0.78 **	0.26 ns	----		
Flower bud count	-0.18 ns	-0.24 ns	-0.16 ns	-0.12 ns	0.17 ns	----	
No. shoots/bulb	-0.18 ns	-0.17 ns	0.37 **	-0.11 ns	0.21 *	0.09 ns	----

a \*, \*\*, \*\*\*, ns denote significant P-values at P < 0.05, P < 0.01, P < 0.001, and not significant, respectively

‘Nellie White’ had 111 leaves under SD and 98 with LD in the greenhouse, but 61 leaves in SD and 106 in LD in the growth chamber (Table 4). Several seed-propagated *L. xformolongi* hybrids had equivalent leaf numbers in both photoperiods, indicating day neutrality (Anderson and Ascher 2001; Erwin and Warner 2002; Anderson and Ascher 2004), e.g. ‘Raizan No. 1’ (Lot#8159) in the greenhouse. Day neutrality is evident as this hybrid flowered in an equivalent amount of time, 222 d (SD) and 223 d (LD) in the greenhouse environment (Table 4). ‘Nellie White’ acts as a quantitative LD plant (98 leaves in LD, but 111 in SD; Erwin and Warner 2002) in the greenhouse environment; the opposite was true (quantitative SD plant) in the growth chamber, however. Leaf unfolding rates were comparable within genotypes across photoperiods and environments, but frequently differed between genotypes, ranging from one to eight leaves unfolded/week (Table 4).

The mean number of flowers in CC ‘Nellie White’ for both environments was higher than that found for all other genotypes and species (Table 4). Flower number was significantly correlated for VBD ( $r = -0.49$ , Table 5), flowering date ( $r = -0.54$ ), and leaf number ( $r = 0.77$ ) in the greenhouse environment; no significant correlations between flower number and any trait was found in the growth cham-

ber environment (Table 5).

Under both environments and photoperiods, all *L. longiflorum*, with the exception of ‘Snow Trumpet’ (SD, greenhouse), and all CC *L. formosanum* bulbs produced only one shoot/bulb. This is in contrast with flowering *L. formosanum* seedlings (SD, greenhouse) and all flowering *L. xformolongi* seedlings, which produced multiple shoots/bulb (Table 4). The number of shoots was significantly correlated for leaf number ( $r = -0.16$ , Table 5) and flower bud count ( $r = -0.23$ ) in the greenhouse environment and for plant height ( $r = 0.37$ , Table 5) and leaf number ( $r = 0.21$ ) in the growth chamber. Broad sense heritability for shoot number were lowest in the greenhouse ( $h^2 = 0.32$ , 95% C.I. = 0.32-0.32) compared with the growth chamber ( $h^2 = 0.54$ , 95% C.I. = 0.52-0.56).

The parental species (all CC bulbs and seedlings, with one exception) did not reach VBD or reflower under either photoperiod (Table 6). The only exception to this was *L. formosanum* seedlings (Lot#502) under LD with 20% which reached VBD in 177d (25.3 weeks) but never flowered before the experiment was terminated (Table 6). Thus, *L. formosanum* may be the source of gene(s) for the reflowering trait, although this happened only under LD and the second shoots cannot be termed day neutral. Both *L.*

**Table 6** Mean percent reflowering or stems with buds (% rosetted), number of days to visible bud date (VBD), number of days to reflowering, plant height (cm), inflorescence length (cm), number of leaves, and number of flowers or flower buds for *Lilium longiflorum* 'Nellie White', 'Snow Trumpet', *L. formosanum*, and their interspecific *L. xformolongi* F1 and BC1F1 hybrids from the greenhouse environment grown under short (SD) and long (LD) day photoperiods during 7/13/2001 – 12/13/2001. Seed lots were pooled within seed-propagated cultivars.

<i>Lilium</i> species	Cultivar, backcross hybrid	% Reflowering or in bud (% rosetted)		No. days to VBD		No. days to reflowering		Plant height (cm)		No. leaves		Inflorescence length (cm)		No. flowers or flower buds	
		SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD
		<i>L. longiflorum</i>	Nellie White	0(0)	0(0)	-- a	-- a	-- a	-- a	-- a	-- a	-- a	-- a	-- a	-- a
	Snow Trumpet	0(0)	0(0)	--	--	--	--	--	--	--	--	--	--	--	--
<i>L. formosanum</i>	Bulbs	0(0)	0(0)	--	--	--	--	--	--	--	--	--	--	--	--
	Seed Lot#502	0(0)	20(0)	--	177	--	--	--	29	--	--	--	39	--	1.0
<i>L. xformolongi</i>	Raizan No. 1	80(20)	100(0)	140	110	120	114	65	64	60	43	6	11	1.0	1.0
	Raizan No. 2	60(40)	80(20)	159	126	-- a	110	59	63	64	52	5	13	1.0	1.0
	Sakigake Raizan	60(20b)	50(50)	--	--	--	--	62	51	49	24	10	9	1.1	1.0
	00L-13	20(80)	25(75)	190	64	-- a	-- a	65	43	70	41	10	14	1.0	2.0
	00L-74	20(80)	40(60)	149	110	-- a	149	45	51	35	24	10	10	1.0	1.0
	00L-96	0(100)	0(100)	--	--	--	--	--	--	--	--	--	--	--	--
	00L-116	0(100)	20(80)	--	139	--	145	--	61	--	69	--	15	--	1.0
	00L-136	0(100)	20(80)	--	167	--	176	--	65	--	59	--	13	--	1.0

a Did not flower before the experiment terminated; flower buds were either 1-2 weeks from anthesis or had aborted.

b20% of the seedlings also died.

*longiflorum* and *L. formosanum* plants or seeds that did not reflower failed to have shoot emergence above the soil line and leaf numbers were nonexistent. Apparently, there is an obligate cold treatment (vernalization) requirement for reflowering. About 20-40% of the *L. longiflorum* and *L. formosanum* plants died under both photoperiods (data not shown).

*L. xformolongi* commercial F<sub>1</sub> hybrids had a reflowering percentage of 60% to 80% under SD and ~50% to 100% in LD (Table 6). All reflowering F<sub>1</sub> hybrid plants had one flower/stem. 'Raizan No. 1' took 120 days (17.1 weeks) under SD and 114 d (16.3 weeks) in LD to reflower (Table 6); 'Raizan No. 2' reflowered in 110 days (15.7 weeks) under LD. No F<sub>1</sub> hybrid had 100% reflowering under both photoperiods. *L. xformolongi* F<sub>1</sub> hybrids segregate for reflowering and a vernalization requirement. Only 'Raizan No. 1' plants grown under LD photoperiods did not segregate for reflowering.

BC<sub>1</sub>F<sub>1</sub>s had a reflowering percentage of 0-20% under SD (Table 6). The % reflowering under LD was 25% to 40%. Hybrids 00L-13 and 00L-74 had the highest reflowering percentage in both photoperiods. No BC<sub>1</sub>F<sub>1</sub> hybrid had 100% reflowering in both photoperiods. Reflowering hybrids had from 1-3 flowers on the second shoot (Table 6). *L. xformolongi* BC<sub>1</sub>F<sub>1</sub> hybrids also segregated for the reflowering trait and a vernalization requirement. BC<sub>1</sub>F<sub>1</sub> hybrid 00L-13 took 190 d (27.1 weeks) to reach VBD under SD and much longer for reflowering. Under LD, it took 64 d (9.1 weeks) to reach both VBD and reflowering (Table 6). Hybrid 00L-74 took 149 d (21.3 weeks) to reach VBD under SD conditions and longer to reflower; under LD, it took 110 d (15.7 weeks) for VBD and 149 d (21.3 wks) to reflower (anthesis). Hybrid 00L-96 did not segregate for the reflowering trait under either photoperiod. Hybrid 00L-116 did not segregate for the reflowering trait under SD, but took 139 d (19.9 weeks) to reach VBD and 145 d (20.7 weeks) to reflower in LD. All of the *L. xformolongi* BC<sub>1</sub>F<sub>1</sub> hybrids had a large percentage of plants that rosetted under both SD and LD. Of notable significance, one hybrid plant, 00L-75-23, had a second shoot in which VBD and flowering occurred on the same day as the initial shoot.

## DISCUSSION

Germination of the identical seed lots used in earlier experiments (Anderson *et al.* 2012) were lower than those reported herein, despite similar germination conditions between the two experiments (Table 2). While seed germination occurred in the recommended temperatures (Dai-Ichi Seed Co. 1999; Sakata Seed Co. 1998-2000), % seed germination had wide ranges in variation between *L. xformolongi* hybrid

and backcross accessions, as well as parental species (*L. formosanum*, *L. longiflorum*) (Table 2). Similar % germination has been reported for *L. formosanum* (Carpenter and Ostmark 1991; Shii 1983; Mynett 1997), *L. xformolongi* (Anderson *et al.* 2010b; Mynett 1997; Roh and Sim 1996; Watanabe 1993), *L. longiflorum* (Mynett 1997), and *L. pumilum* (Chojnowski and Mynett 1992). Future research is required to elucidate the cause(s) of variation within and among *L. xformolongi* seed lots and backcrosses observed herein (Table 2). Anderson *et al.* (2011) suggested that secondary dormancy (ecodormancy) might be a causal factor. It may be possible to select against this trait by continued backcrossing and inbreeding hybrids with high germination rates, e.g. 00L-74 and 00L-116 (Table 2), to concentrate favorable alleles. Since yield potential for these two BC<sub>1</sub>F<sub>1</sub>s were also high, maintaining this potential linkage would be desirable.

Variation in yield potential differences also occurred between and within seed lots (Table 2). Most were less than the % yield potential values for commercial seed crops (PanAmerican Seed Co. 2005), with exception of 'White Lancer' (75%), 'Snow Trumpet' (73.5%), 00L-116 (80%), and 'Raizan No. 1' Seed Lot No. 8241 (72%) (Table 2). Similar findings were reported in many of the same (Anderson *et al.* 2012) or different (Roh and Sim 1996) cultivar seed lots. Anderson *et al.* (2011) proposed that directed selection and improvement could increase yield potential. Indeed, the backcross progeny—the direct result of such selection—demonstrates the potential to achieve directed genetic improvement (BC<sub>1</sub>F<sub>1</sub> hybrids, Table 2).

Non-vernalized (non-CC) *L. longiflorum*, *L. formosanum* lily bulbs did not develop flowers under either LD or SD conditions (Table 4), similar to previous experiments (Anderson *et al.* 2012). Vernalized (CC) *L. longiflorum* and *L. formosanum* bulbs flowered in both photoperiod treatments under greenhouse conditions. In the case of *L. longiflorum* 'Nellie White', SD-treated bulbs required several days or weeks longer than LD to reach VBD and flowering reconfirming the quantitative LD response for flower bud initiation at ≤ 21°C (Anderson *et al.* 2010b; Lange and Heins 1988; Roh and Wilkins 1973). In contrast with the greenhouse environment, CC 'Nellie White' bulbs did not reach VBD nor flower under SD in the growth chamber (Table 4), even though the temperatures were cooler than in the LD photoperiod (growth chamber). Likewise, the greenhouse temperatures exceeded the tighter control in the growth chamber. Seed-propagated *L. longiflorum* 'Snow Trumpet' and *L. formosanum* (Seed Lot #502) did not reach VBD nor flower under either photoperiod in the growth chambers while both behaved as quantitative LD plants in the greenhouse environment (*cf.* no. days to VBD and





**Fig. 1** Flowering of seed-propagated *Lilium xformolongi* backcross hybrid BC<sub>1</sub>F<sub>1</sub> 00L-116 under long day (left; plant No. 15) and short day (right; plant No. 13) photoperiod treatments in the greenhouse.

flowering, as well as dissimilar leaf numbers between photoperiods in the same environments, **Table 4**). It is plausible the ‘Snow Trumpet’ has a different temperature threshold for flower bud initiation than ‘Nellie White’ (21°C; Roh and Wilkins 1973), although this has not been reported. VBD and flowering response of *L. longiflorum* ‘Snow Trumpet’ and *L. formosanum* (Seed Lot #502) are in contrast with *L. formosanum* CC bulbs which reached VBD in all photoperiods in both environments, i.e. 1 week earlier under SD in the greenhouse and only 2 d earlier under LD in the growth chambers, despite having different mean leaf numbers (**Table 4**). CC *L. formosanum* bulbs are ~day neutral for flower bud initiation and early flower bud development, but not for later flower bud development since they did not flower under LD in either environment (**Table 4**) – despite the difference in leaf number between photoperiods, as is the norm (Erwin and Warner 2002). However, due to insufficient bulb numbers, non-CC bulbs of *L. formosanum* were not tested, preventing elucidation of the vernalization requirement (obligate, facultative, or non-obligate). Also, the duration and vernalization temperature for *L. formosanum* bulbs has not been reported (Watanabe 1993) and may differ from *L. longiflorum* ‘Nellie White’ (De Hertogh 1996). Thus, in the current experiments *L. formosanum* bulbs received the same duration and temperature for vernalization as *L. longiflorum*. Since the seed-propagated *L. formosanum* responded differently than CC bulbs, considerable genetic variation within this parental species exists for flower bud initiation and development requirements (Mynett 1997); the same is true for *L. longiflorum*.

Rapid flower bud initiation (VBD in 127 d – 274 d from sowing, **Table 4**) and development (flowering in 217 d – 306 d, **Table 4**; cf. ‘extreme early flowering’, Hiramatsu *et al.* 2012) of seed-propagated *L. xformolongi* hybrids and backcrosses without vernalization (**Fig. 1**) indicates a short juvenility period (Fukai *et al.* 2005; Rhee *et al.* 2005; Hiramatsu *et al.* 2012). Previous reports of flowering seed-propagated *L. xformolongi* were similar (Anderson *et al.* 2012) or exceeded the current findings (270 d; Mynett 1997), if they were quantified (Shii 1983 did not quantify flowering data). The only hybrid to exceed Mynett’s (1997) 270 d period was BC<sub>1</sub>F<sub>1</sub> 00L-136 under SD in the greenhouse (**Table 4**). Whether BC<sub>1</sub>F<sub>1</sub> 00L-136 also possess dominant alleles for *VER1* and/or *VER2* identified in *L. formosanum* (Zlesak and Anderson 2010) is unknown. *L. formosanum* is derived from *L. longiflorum* (Hiramatsu *et al.* 2012), backcrossing to either parental species, rather than just *L. formosanum*, may not impede progress in concen-

trating favorable alleles for early flowering. Since BC<sub>1</sub>F<sub>1</sub> 00L-136 did not also have high seed germination and yield potentials (**Table 2**), directed breeding and selection would be essential to incorporate these as a suite of traits important for plug production and flowering.

It is unknown at the present time whether one or several seed-propagated hybrid cultivar(s) could be used for Easter potted or cut flower production since this holiday varies in dates during March-April of each calendar year (Zlesak and Anderson 2010). If a hybrid could be selected for early flowering and, likewise, be stable across environments for this and other critical traits (germination, yield potential, day neutrality, leaf number, plant height, flower bud count) its flowering could be regulated by sowing date and forcing temperature. This would allow it to be forced for varying Easter dates. However, given the wide range in variation for all traits examined (**Tables 2-6**), this may be an overly ambitious breeding objective and greater progress could be realized with the development of hybrids to flower across the range of Easter holiday weeks, e.g. early, mid, and late Easters.

Early-flowering hybrids, such as ‘Raizan No. 2’ (Lot#8086, LD, growth chamber, **Table 4**), would have value as seed-propagated flowering potted plants, bedding plants, as well as cut flowers (the current use in Japan, Korea) (Rhee *et al.* 2005). The lack of stability in time to VBD and flowering within the greenhouse environment, as evidenced by the hybrid seed lots used in this experiment (**Table 4**) and previously (Anderson *et al.* 2012), indicates genotype × environment (G×E) interactions. Stability (lack of G×E) would be a requirement to ensure programmable commercial production of seed-propagated hybrids as flowering potted plants for Easter sales or cut flowers throughout the year (Anderson 2000, 2003). Since all of the BC<sub>1</sub>F<sub>1</sub> hybrids failed to flower at all in the growth chamber environment (**Table 4**), significant G×E also exists between environments. Stability for VBD and flowering, as well as other critical traits, will be essential for world-wide production at varying latitudes.

Day neutral species with equivalent leaf numbers under both SD and LD (Erwin and Warner 2002) occurred in several seed-propagated *L. xformolongi*. ‘Raizan No. 2’ (Lot #8086, Lot #8148, **Table 4**) and BC<sub>1</sub>F<sub>1</sub> 00L-136 had nearly identical leaf numbers in the greenhouse environment and flowered in both photoperiods indicating day neutrality (Anderson and Ascher 2001, 2004). However, the numbers of days to flowering differed between these seed lots, genotypes, and photoperiods making these either facultative SD or facultative LD plants. Such wide variation both within and among backcross hybrids will need to be removed with inbreeding and directed selection. Both ‘Raizan No. 1’ (Lot#8159) and ‘Raizan No. 2’ (Lot#8148) had higher leaf numbers in the Anderson *et al.* (2011) experiment than in the present one (**Table 4**), whereas the leaf numbers in ‘Sakigake Raizan’ (Lot#9097) were comparable (stable) between the two experiments. Photoperiod was significant for VBD, flowering, and leaf numbers (**Table 3**) although such significant differences may be due, in part, to the inclusion of *L. longiflorum* and *L. formosanum* vernalized/non-vernalized bulbs (**Table 4**). Thus, a lack of stability or G×E interactions was evident for day neutrality (flowering) and leaf number. Shoot emergence in *L. formosanum* populations was day-neutral, while internode lengths, leaf unfolding rates, and flower bud initiation were facultative LD responses (Shii 1983).

*L. xformolongi* reflowered in the greenhouse (**Table 6**), similar to *L. formosanum* (Shii 1983; Walters 1983) and *L. xformolongi* (Anderson *et al.* 2012), earlier (114 d for ‘Raizan No. 1’; 110 d for ‘Raizan No. 1’, **Table 6**) than the initial flowering (254d-SD, 229d-LD for ‘Raizan No. 2’; 222d-SD, 223d-LD for ‘Raizan No. 1’, **Table 4**). It should be noted that reflowering of the second shoot (**Table 6**) did not follow the same flowering sequence across cultivars noted for the first shoot (**Table 4**). Likewise, reflowering of the second shoot varied for vernalization requirements

(obligate vs. non-obligate), e.g. reflowering of *L. xformolongi* hybrids was < 100% in all cases (Table 6), similar to *L. formosanum* populations (McRae 1988; Wall 1997). It is unclear why expression of the *L. formosanum* *VER1*, *VER2* genes (Zlesak and Anderson 2010) in *L. xformolongi* would differ for vernalization requirement between the first and second shoots and whether or not this also occurs in *L. formosanum*. This would need to be clarified and selected in advanced inbreds and hybrids before they could be forced as continuous flowering products.

Leaf number was negatively correlated with the number of days to VBD ( $r = -0.45$ ,  $P \leq 0.01$ , greenhouse;  $r = -0.03$ , n.s., growth chamber, Table 5), the opposite of what was found previously (Anderson *et al.* 2012). Similarly, there were differences in *L. xformolongi* response both experiments for leaf number correlations with plant height. 'Raizan No. 1' (Lot#8159), 'Raizan No. 2' (Lot#8148), and 'Sakigake Raizan' (Lot#9097) had higher leaf unfolding rates (Anderson *et al.* 2012) unlike the current findings in the greenhouse (Table 4). Typically, higher leaf numbers are correlated with increased plant height since more leaves need to be 'laid down' prior to VBD as in *L. longiflorum* (De Hertogh 1996). Flowering date and leaf number were significantly correlated in all environments and experiments, but differed for positive vs. negative effects (Table 5).

Leaf number was highly heritable in the greenhouse ( $h^2 = 0.83$ ) and growth chamber ( $h^2 = 0.93$ ) environments. In the greenhouse, plant height of the *L. formosanum* CC bulbs, BC<sub>1</sub>F<sub>1</sub> 00L-136, 00L-96, 00L-74, and 00L-13 all were statistically similar to 'Nellie White' CC bulbs, whereas *L. formosanum* seedlings (Lot#502) and the remaining genotypes were not (Table 4). The genotypes similar to 'Nellie White' may be the most likely source(s) for short stature seed-propagated lilies for Easter sales. It is encouraging that backcrosses between 'Nellie White' (female) and *L. xformolongi* hybrids (BC<sub>1</sub>F<sub>1</sub> 00L-136) were as short as 'Nellie White' in the greenhouse environment (Table 4). In the other BC<sub>1</sub>F<sub>1</sub> hybrids with 'Nellie White' as the male parent, plant height overlapped with or exceeded that of 'Nellie White' (Table 4). Thus, to breed and select for seed-propagated Easter lilies, careful selection of potential *L. xformolongi* germplasm and the cytoplasmic parent would be important. In cases where hybrids exceed desirable plant heights for potted Easter lilies, the application of plant growth regulators and/or DIF might be feasible to create a salable product (Dole and Wilkins 2005).

## CONCLUSION

In this work, the author compares different bulb and seed populations of hybrids (*L. xformolongi*), BC<sub>1</sub>F<sub>1</sub> and the progenitors. Regardless of photoperiod or environment, seed-propagated *L. xformolongi* flowered in <1 year from sowing and often reflowered without cold whereas non-vernalized *L. formosanum*, *L. longiflorum* bulbs never flowered in SD or LD across environments. Traits such as VBD, flowering date and leaf number were highly heritable. One BC<sub>1</sub>F<sub>1</sub> was day-neutral for flowering. Two *L. xformolongi* BC<sub>1</sub>F<sub>1</sub> produced significantly less leaves than 'Nellie White' whereas plant height in several BC<sub>1</sub>F<sub>1</sub> did not differ from 'Nellie White'. BC<sub>1</sub>F<sub>1</sub> flowered in the greenhouse environment but did not flower in the growth chambers, indicating significant GxE interactions. The wide range of genetic variation in the BC<sub>1</sub>F<sub>1</sub> for all traits examined could be attributed to backcrossing with non-inbred 'Nellie White' as well as *L. xformolongi* parents. Further inbreeding and backcrosses are required to concentrate favorable alleles in select parents with high combining ability before hybrid seed lines can be selected which are stable across plug and finishing production environments (minimal GxE), have high seed germination and yield potentials, are day neutral for critical flowering traits (VBD, anthesis), produce low leaf numbers, have reduced plant height (for potted plant production), initiate and develop high flower bud counts, and reflower on a continual basis.

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## REFERENCES

- Anderson NO (2000) Forcing requirements of seed-propagated Easter lilies. *HortScience* 35 (3), 470 (Abstract)
- Anderson NO (2003) *L. xformolongi* lilies. Fish & Richardson, Document No. 09531-036P01 / Z01062. U.S. Utility Patent Application No. 20040003440. U.S. Patent Office, Washington, D.C. Available online: <http://www.uspto.gov/> and <http://www.freepatentsonline.com/y2004/0003440.html>
- Anderson NO, Ascher PD (2001) Selection of day-neutral, heat-delay-insensitive *Dendranthema xgrandiflora* genotypes. *Journal of the American Society for Horticultural Science* 126 (6), 710-721
- Anderson NO, Ascher PD (2004) Inheritance of seed set, germination, and day neutrality/heat delay insensitivity of garden chrysanthemums (*Dendranthema xgrandiflora*) under glasshouse and field conditions. *Journal of the American Society for Horticultural Science* 129 (4), 509-516
- Anderson NO, Dunn B (2003) Inbreeding depression in seed-propagated *Lilium x-formolongi* inbreds. *Acta Horticulturae* 624, 43-49
- Anderson NO, Younis A, Sun Y (2010) Intersimple sequence repeats distinguish genetic differences in Easter Lily 'Nellie White' clonal ramets within and among bulb growers over years. *Journal of the American Society for Horticultural Science* 135 (5), 445-455
- Anderson NO, Berghauer E, Harris D, Johnson K, Lonroos J, Morey M (2012) Discovery of novel traits in seed-propagated *Lilium*: non-vernalization-requiring, day-neutral, reflowering, frost-tolerant, winter-hardy *L. xformolongi* I. Characterization. In: Van Tuyl J, Arens P (Eds) *Bulbous Ornamentals. Floriculture and Ornamental Biotechnology 6 (Special Issue 2)*, 63-72
- Carpenter WJ, Ostmark ER (1991) Handling, storage, and germination of Formosan lily seed. *Proceedings, Annual Meeting Florida State Horticultural Society* 103, 209-212
- Chojnowski M, Mynett K (1992) Germination of *Lilium pumilum* seeds. *Acta Horticulturae* 325, 235-238
- Dai-Ichii Seed Company (1999) '99 Dai-Ichii Seed. Minato-Ku, Tokyo, Japan, 17 pp
- De Hertogh AA (1996) *Holland Bulb Forcer's Guide* (5<sup>th</sup> Edn), Alkemade Printing BV, Lisse, The Netherlands, 300 pp
- Dole JM, Wilkins HF (1996) Directions of *Lilium* research. *Acta Horticulturae* 414, 295-300
- Dole JM, Wilkins HF (2005) *Floriculture: Principles and Species* (2<sup>nd</sup> Edn), Prentice Hall, Upper Saddle River, NJ, 1040 pp
- Erwin JE, Engle-Egiles G (1998) Influence of simulated shipping and rooting temperature and production year on Easter lily (*Lilium longiflorum* Thunb.) development. *Journal of the American Society for Horticultural Science* 123, 230-233
- Fukai S, Isoe T, Ikoma C (2005) LLO hybrid lilies from *L. xformolongi* and chromosome doubled *L. formosanum* var. *pricei* x Oriental hybrid 'Le Reve'. *Acta Horticulturae* 673, 377-381
- Hiramatsu M, Masuda J-I, Sakazono S, Okubo H (2012) Evolution of early flowering ability specific to *Lilium formosanum* from its progenitor species *L. longiflorum*. In: Van Tuyl J, Arens P (Eds) *Bulbous Ornamentals. Floriculture and Ornamental Biotechnology 6 (Special Issue 2)*, 21-27
- Lange NE, Heins R (1988) Interaction of photoperiod and temperature in promoting flowering of *Lilium longiflorum*. *HortScience* 23, 749
- Lin WC, Wilkins HF (1973) The interaction of temperature on photoperiodic responses of *Lilium longiflorum* Thunb. cv. 'Nellie White'. *Florist's Review* 13 (3965), 24-26
- McRae EA (1998) *Lilies: A Guide for Growers and Collectors*, Timber Press, Portland, OR, 392 pp
- Mynett K (1992) Twice a year flowering of *Lilium* 'Prima'. *Acta Horticulturae* 325, 355-356
- Mynett K (1997) Badania nad przebiegiem kwitnienia *Lilium formosanum* Wallace, *L. longiflorum* Thunb. i ich miedzygatunkowego mieszańca *L. x formolongi* w nie ogrzewanej szklarni. *Zeszyty Problemowe Postępów Nauk Rolniczych* 449, 125-134
- Okazaki K (1996) *Lilium* species native to Japan, and breeding and production of *Lilium* in Japan. *Acta Horticulturae* 414, 81-92
- PanAmerican Seed Co. (2005) *Product Information Guide*, W. Chicago, IL, np
- Prince T, Prince T (2003) Easter lily demand. *Greenhouse Grower*, March Issue, 50, 52, 54
- Rhee HK, Lim JH, Kim YJ, van Tuyl JM (2005) Improvement of breeding efficiency for interspecific hybridization of lilies in Korea. *Acta Horticulturae* 673, 107-112
- Roh SM, Sim YG (1996) Seed germination of *Lilium x formolongi* as influenced by temperature and plant growth regulators. *Acta Horticulturae* 414,

243-250

- Roh SM, Wilkins HF** (1973) The influence and substitution of long days for cold treatments on growth and flowering of Easter lilies (*Lilium longiflorum* Thunb. 'Georgia' and 'Nellie White'). *Florists' Review* **153** (3960), 19-21, 60-63
- Sakata Seed Co.** (1998-2000) *Sakata's Reliable Seed: Flower Seeds*, Yokohama, Japan, 58 pp
- Shii CT** (1983) The distribution and variation of *Lilium formosanum* Wall. and *L. longiflorum* Thunb. in Taiwan. *Yearbook of the North American Lily Society* **36**, 48-51
- Stuart NW** (1954) Moisture content of packing medium, temperature and duration of storage as factors in forcing lily bulbs. *Proceedings of the American Society for Horticultural Science* **63**, 488-494
- United States. Department of Agriculture. Foreign Agricultural Service** (2011) *Global Agricultural Information Network (GAIN) Report: Japan, Niigata Report 2011*. GAIN Report No. JA1515. Available online: [http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Niigata%20Report%202011\\_Tokyo%20ATO\\_Japan\\_11-2-2011.pdf](http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Niigata%20Report%202011_Tokyo%20ATO_Japan_11-2-2011.pdf)
- Wall J** (1997) *L. formosanum*-Growing wild in Florida. *North American Lily Society Quarterly* **51**, 18-23
- Walters G** (1983) Naturalization of *Lilium formosanum* in South Africa. *Yearbook of the North American Lily Society* **36**, 44-47
- Watanabe H** (1993) Shinteppoyuri (*L. x formolongi*). In: Kunishige M (Ed) *Lilium – Breeding and Culture*, Seibundo Shingosha, Japan, pp 172-179
- Weiler TC, Langhans RW** (1968) Determination of vernalization temperatures in the vernalization requirement of *Lilium longiflorum* (Thunb.) cv. 'Ace.' *Proceedings of the American Society for Horticultural Science* **93**, 623-629
- Weiler TC, Langhans RW** (1972) Growth and flowering responses of *Lilium longiflorum* Thunb. 'Ace' to different daylengths. *Journal of the American Society for Horticultural Science* **97**, 176-177
- Wilkins HF** (1973) Our Easter lily: Where did it come from, why does it flower at Easter time; chasing the wild lily. *Minnesota Horticulture* **101**, 36-38
- Zlesak D, Anderson N** (2003) Inside west coast Easter lily production. *Minnesota State Florists' Bulletin* **52**, 4-6
- Zlesak D, Anderson N** (2010) Inheritance of non-obligate vernalization requirement for flowering in *Lilium formosanum*. In: Kamenetsky R (Ed) *Ornamental Geophytes: From Basic Science to Sustainable Horticultural Production*. *Israel Journal of Plant Sciences* **57** (4) **Special Issue**, 315-327