

Discovery of Novel Traits in Seed-Propagated *Lilium*: Non-vernalization-requiring, Day-neutral, Reflowering, Frost-tolerant, Winter-hardy *L. xformolongi*. I. Characterization

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ABSTRACT

Lilium are important floricultural crops worldwide. The objectives of this research were to examine *L. xformolongi* hybrids and a parental species (*L. longiflorum*) in photoperiods (Exp. 1) and environments (greenhouses, Exp. 1; field, Exp. 2) for flowering without vernalization, post-emergence photoperiod, potted plant/field performance, frost tolerance, and winter hardiness. Seed germination (4-15%) and yield potential (3.5-12.5%) varied between genotypes. In Exp. 1, short/long days had a significant effect on visible bud date only, but not on leaf unfolding rates, plant height, leaf number, or flowering dates; cultivar differences were highly significant. Cultivar × photoperiod interactions were nonsignificant except for flowering date ($P = 0.04$). ‘Nellie White’ (case-cooled bulbs) flowered in 213 d, while *L. xformolongi* cultivars took 247 d (‘Sakigake Raizan’) to 306 d (‘Raizan No. 3’) from sowing. Both VBD ($h^2 = 0.93$) and flowering date ($h^2 = 0.91$) were highly heritable (Exp. 1) and correlated. Regardless of photoperiod and environment, *L. xformolongi* flowered in <1 yr from sowing without cold. Leaf number ($h^2 = 0.81$, Exp. 1) and unfolding rates ($h^2 = 0.93$) were not as tightly linked in *L. xformolongi* as ‘Nellie White’. No seed-propagated hybrids (98-164 cm) were as short as ‘Nellie White’ (62 cm). ‘Augusta F₁’ had the highest flower bud counts (6.9 / plant). Shoot numbers ranged from $n=1$ (‘Nellie White’) to $n = 3.8$ (‘Sakigake Raizan’). *L. xformolongi* reflowered continuously in the field, but varied for frost-tolerance (25-75%; Exp. 2). Winter survival ranged from 0 to 87.5% in *L. xformolongi* over two years (Exp. 2). Flowering and reflowering of seed-propagated *L. xformolongi* in <1 yr. without vernalization, frost-tolerance, day neutrality, and winter hardiness are novel trait combinations for *Lilium*.

Keywords: day neutrality, flowering, heritability (broad-sense), leaf number, *Lilium longiflorum*, photoperiodism, visible bud date
Abbreviations: CC, case-cooled; CI, confidence interval, LD, long day; SD, short day; VBD, visible bud date

INTRODUCTION

The genus *Lilium* (lilies) comprises ~85+ species (Asano 1986, 1989; de Jong 1974; Huxley *et al.* 1992) native to the northern hemisphere, occurring primarily between 10 and 60° latitudes (Anderson 1986). Several horticultural lily groups are important commercially, i.e. trumpets (particularly Longiflorums, L), Asiatics (A), Orientals (O), Martagons, and LA, OT, Aurelian hybrids. Easter lily (*Lilium longiflorum* Thunb.) is the best-known trumpet lily, commercially grown in N. America as flowering potted plants for Easter (Dole and Wilkins 2005). Elsewhere lily cultivars are favored as cut flowers, including ‘Snow Queen’ (Europe), ‘Gelria’ (Europe), ‘White American’ (Europe), ‘Osnat’ (Israel, Europe), and ‘Shovav’ (Israel). Potted Easter lilies (‘Nellie White’) rank in the top ten flowering potted plants sold in the United States, with 6.25 million pots sold at a US\$27.316 million (wholesale) farm-gate value in 2009 (United States Department of Agriculture National Agricultural Statistics Service 2010). Since the Easter lily market is two weeks prior to Easter (with a variable date each year), growers must precisely control growth and flowering of this crop to meet the holiday (De Hertogh 1996; Dole and Wilkins 2005). Prince and Prince’s (2003) survey of age groups purchasing potted Easter lilies projected an increased market demand over the next ten years. Response to this demand will require increased production.

Easter lily propagation and field production for the N. American market occurs in a small coastal region between Smith River, CA and Brookings, OR (Wilkins 1973, 1980). During World War II, Easter lily production commenced in

this coastal region. Japan had been the major Easter lily source for the N. American market (Clark 1949). The three U.S. cultivars since 1945 have been ‘Ace’, ‘Croft’, and ‘Nellie White’; the latter is the only one currently in production (Clark 1949). This narrow genetic base may jeopardize long-term crop viability (Anderson *et al.* 2010).

Easter lily production in the U.S. is very different from other floriculture crops, due to a narrow germplasm base with the production of one cultivar (‘Nellie White’), no central propagator or distributor, and production in a small geographical region (Zlesak and Anderson 2003a). Each bulb grower selects their own clonal variant(s) of ‘Nellie White’ (H. Harms 2000, pers. comm.), a common practice for many commercial lily groups. Phenotypic and genetic variation within this clone necessitates purchasing ‘Nellie White’ from several different bulb growers to ensure flowering for Easter (Wilkins 1980; Erwin and Engelen-Egles 1998; Zlesak and Anderson 2003b; Anderson *et al.* 2010).

Lily species and cultivars are also classified into four categories for the time of flower bud initiation and development (Baranova 1972; Ohkawa *et al.* 1990): (I) flower buds initiate in late summer and continue to develop into late autumn (early flowering Asiatic and Oriental cultivars; ~31% of Asiatic types are in this category), (II) flower bud initiation occurs in later summer although flower bud development is not completed until the following spring (Asiatic and Oriental species, e.g. *L. rubellum*), (III) flower bud initiation and development occurs in the spring prior to shoot emergence above the soil (most Asiatic and Oriental cultivars), and (IV) flower bud initiation and development

occurs in the spring after shoot emergence (Asiatics – later flowering types ~69%, Easter lily and most other trumpets and some Orientals). All *Lilium* species and cultivars have an obligate requirement of an extended cold treatment (vernalization) for rapid stem elongation and emergence above the soil (De Hertogh 1996; Dole and Wilkins 2005).

Lilies have underground storage organs (imbricated, nontunicate bulbs – a configuration of fleshy scales stacked like bricks) and are classified as geophytes (Blaney and Roberts 1967). A commercial 2-3 year old bulb has inner or daughter scales (from the most recent growing season), outer or mother scales (>1 year old; from the previous growing season), and an apical meristem (growing point) with leaves (Blaney and Roberts 1967; Dole and Wilkins 2005). The inner scales have not been exposed to a cold period (winter) and contain inhibitors which prevent stem elongation and shoot emergence without a cold (vernalization) treatment (Dole and Wilkins 1996; Roh 1999). Commercial potted and cut lily cultivars in all horticultural classes are predominantly vegetatively-propagated (bulbs, bulb divisions, aerial axillary stem bulbils), rather than sexually (seed) (Beattie and White 1993; Griffiths 1934).

Easter lilies have an obligate vernalization requirement of six wks or 1,000 h at 2-7°C in moist conditions (Stuart 1954). If *L. longiflorum* bulbs do not receive the required duration of cooling at 2-7°C and are grown at temperatures >21°C they will not flower (Lin and Wilkins 1973). Bulbs are vernalized either in the shipping cases (case-cooled), in pots after rooting for 1-3 weeks at 17-18°C (controlled temperature forcing, CTF), or naturally in pots placed out-of-doors (after rooting) at 1-9°C (De Hertogh and Wilkins 1971a, 1971b; Dole and Wilkins 2005).

In the Ryuku Islands (27° N lat.) where *L. longiflorum* are native, wild Easter lilies flower after long days (LD) and a short duration of temperatures <21°C (Wilkins 1973). If insufficient vernalization (<6 weeks at 2-7°C) is applied to either case-cooled, CTF, or natural cooled lily bulbs, LD photoperiods (≥12 h) can provide an equal substitution for the lack of cold after shoot emergence (Walters and Wilkins 1967; Weiler and Langhans 1968, 1972; Wilkins and Roh 1970; Lange and Heins 1988). Easter lilies will eventually flower if exposed to short days (SD) after vernalization and shoot emergence, but the flowering date may be too late, particularly when Easter is early. Consequently, growers institute post-emergence LD treatments when Easter is early. Thus, Easter lilies are quantitative LD plants at the post-shoot emergence stage, which means they will initiate flower buds faster under LD than with SD.

Seed-propagated white trumpet lilies, *L. xformolongi* Hort. (= *L. xformolongo*; Dai Ichii Seed Co. 1999; Sato *et al.* 2010) were introduced for commercial cut flower production in the 1990s. This interspecific hybrid is the product of the cross *L. formosanum* Wallace × *L. longiflorum* (Ascher and Peloquin 1968; Hiramatsu *et al.* 2002) and was first created by Nishimuru in ~1928 (Okazaki 1996). Both parental species are diploid (2n=2x=24) and in the section *Leucolirion* (Hiramatsu *et al.* 2002; Dai *et al.* 2007) with minor cytological differences (Smyth *et al.* 1989). *Lilium xformolongi* cut flower production encompasses ~50 ha in Japan with ~15 million stems/year in 1996 (Okazaki 1996) and 12 million in 2006 (Ho *et al.* 2006). Recent cut lily production statistics for Japan indicate that the total planted area is 165 ha, producing 17.2 million stems in 2011 (U.S. Dept. of Agriculture. Foreign Agricultural Service 2011). Cut flower production is a one-time harvest (1 stem/seedling; Goo and Kim 2008). Commercial *L. xformolongi* cultivars include ‘Augusta F₁’, ‘Azusa’, ‘Bailansa’, ‘Hakuryu’, ‘Kitazawa-Wase’, ‘Norikula’, ‘Raizan’ (Nos. 1-3), ‘Raizan Early’, ‘Sakigake Raizan’ (= ‘Raizan Herald’), and ‘Snow Line’ (Mii *et al.* 1994; Roh and Sim 1996; Dai Ichii Seed Co. 1999; Rhee *et al.* 2005; Ho *et al.* 2006; Goo and Kim 2008; Amaki and Takeuchi 2009; Sato *et al.* 2010; Liu *et al.* 2011). These cultivars flower within 1 yr from sowing (Sato *et al.* 2010; Hiramatsu *et al.* 2012), have the characteristic of wide leaves and showy flowers of *L. longiflorum* (Oka-

zaki 1996; Hiramatsu *et al.* 2012), but are susceptible to virus (McRae 1998). Ho *et al.* (2006) reported that seed-propagated hybrids had high variability.

The development of day-neutral, non-vernalization-requiring lilies (Dole and Wilkins 1996) that are seed propagated is desirable (Griffiths 1934). Seed-propagated *L. xformolongi* may offer such an opportunity, if they possess such traits. It is unknown, however, whether such traits exist or if these could be incorporated into commercial lily production. The objectives of this research were to examine *L. xformolongi* hybrids and a commercial parental species (*L. longiflorum*) for flowering without vernalization, post-emergence photoperiodic requirements, garden performance, and winter hardiness.

MATERIALS AND METHODS

Germplasm

Seeds of *L. xformolongi* F₁ hybrids ‘Augusta F₁’ (Seed Lot No. 9149), ‘Raizan No. 1’ (MAFF Reg. #2835; Seed Lot No. 8159; also termed ‘Raizan No. 1’, Liu *et al.* 2011), ‘Raizan No. 2’ (Seed Lot No. 8148), ‘Raizan No. 3’ (MAFF Reg. #2836; Seed Lot No. 8418), and ‘Sakigake Raizan’ (= ‘Raizan Herald’; Seed Lot No. 9097) were obtained from Dai-Ichii Seed Co., Ltd. (Tokyo, Japan) in 1999. ‘Sakigake Raizan’ flowers 2-3 weeks earlier than ‘Raizan No. 1’ (the first seed-propagated upright lily) (Dai-Ichii Seed Co. 1999). ‘Raizan No. 2’ flowers 3-4 weeks later than ‘Raizan No. 1’ while ‘Raizan No. 3’ is 2 weeks later than ‘Raizan No. 2’. ‘Augusta F₁’ is a uniform flowering hybrid, described to flower between ‘Raizan No. 1’ and ‘Raizan No. 2’ (Dai-Ichii Seed Co. 1999).

Field-dug bulbs of *L. longiflorum* ‘Nellie White’ (22.9-25.4 cm or 9-10” circumference), a parental species, were donated by Ollie Hoffman, Fred Gloeckner Co. (Harrison, New York, U.S.A.) after fall digging in October 1999. These served as comparisons for the seed-propagated material since no commercially available seed of either *L. formosanum* or *L. longiflorum* could be obtained during 1999.

Experiment No. 1. Photoperiod – Greenhouse

Seeds (n = 200/hybrid) were sown on 9 July 1999 in 288 plug trays with germination mix (Sunshine Mix #3, SunGro Horticulture, Bellevue, WA, USA), covered lightly with finely milled sphagnum moss peat, and germinated under mist (5 sec/3 min) at 18/15°C day/night (Shii 1983). The recommended germination temperature for *L. xformolongi* is 15-18°C (Dai-Ichii Seed Co. 1999). Germination counts were performed every 7 d for 8 wks. After germination ceased, plug trays were moved to the glasshouse 23/21°C day/night under natural daylength conditions (long days, 45°N lat., St. Paul, MN USA) until 1 Sept 1999. When true leaves were visible on 10 Sept. 1999, seedlings were transplanted into 21-cm pots using Premier® Pro-Mix® BX (Premier Horticultural Inc., Quakertown, PA) and supplemental lighting was provided for LD (0900-1600 HR, 100 μmol s⁻¹ m⁻²). The seedlings were fertilized weekly with 100 ppm N supplied with 20-10-20 (Plantex, Plant Products Co., Ltd., Leamington, Ontario, Canada) and monthly fungicide drenches. As the seedlings grew, biweekly soil tests were performed and fertilization was adjusted accordingly. No plant growth regulators were applied during this experiment. Pots were spaced 48 cm O.C. in a completely randomized design (CRD) with n = 10 reps. One subsequent transplanting into 36 cm standard pots occurred in week 20, maintaining the original CRD design; spacing was adjusted to 72 cm O.C. Photoperiod treatments of short day (SD; 0800-1600 HR, 100 μmol s⁻¹ m⁻²) and LD (0800-1600 HR, 100 μmol s⁻¹ m⁻² + night interruption lighting 2200-0200 HR, 2 μmol s⁻¹ m⁻²) commenced on 8 Oct. 1999 to test for day neutrality (Anderson and Ascher 2004). Photoperiodic treatments continued until all seedlings flowered in 2000.

Concurrently, one case of field-dug bulbs of *L. longiflorum* ‘Nellie White’ were received on 29 Oct. 1999 (= 101 d after sowing *L. xformolongi* seeds). Upon receipt, the bulbs were treated as case cooled (CC) bulbs for vernalization (De Hertogh 1996). The CC bulbs were placed in a walk-in cooler in the

shipping crate buried in moist moss peat where they received 1,000 hr or six wks of cooling at 4.4°C (De Hertogh 1996). At the end of the vernalization treatment, the CC bulbs (n = 74) were potted into 36 cm standard pots with Universal soil medium (SB300, SunGro Horticulture, Bellevue, WA, USA), moved to the greenhouse containing the seedlings and divided into two equal groups assigned to the LD or SD photoperiods already in progress. To ensure that at least the LD treated bulbs initiated flower buds post-emergence on or near 28 Jan 2000, n = 10 extra sample bulbs/photoperiod were examined under a dissecting microscope; the total number of leaves to be laid down prior to visible bud date (VBD) were counted (De Hertogh 1996).

The following data were collected weekly or daily for the seed-propagated *L. xformolongi* and vegetative *L. longiflorum*, as appropriate: seed germination (weekly counts), % germination, % yield potential, VBD, leaf unfolding rate/wk (calculated as the total number of leaves unfolded/no. of wks VBD; Wilkins 1980), plant height from the soil surface (cm), number of leaves, flower bud count, flowering date, and number of shoots/bulb. Percent germination [$(\Sigma \text{ no. seeds germinating} / \Sigma \text{ no. seeds sown}) \times 100$] and yield potential [$(\Sigma \text{ transplanted seedlings} / \Sigma \text{ no. seeds sown}) \times 100$] (Anderson and Ascher 2004; PanAmerican Seed Co. 2005) were calculated. The commencement date for seed sowing (7 July) and the receipt date for the bulbs (29 Oct.) were used as Day 0, respectively, since both were targeted to flower as close to Easter as possible. Data were analyzed using the Statistical Package for the Social Sciences (SPSS; University of Chicago, Chicago, IL, USA). Analyses of Variance were performed, as well as mean separations using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$. Non-parametric statistical tests (Kruskal-Wallis χ^2 for traits which violated ANOVA assumptions (flower bud counts, number of shoots/bulb) were grouped by photoperiods (SD vs. LD) to test for significance.

Broad sense heritability (h^2) estimates (entry-mean basis) were calculated for VBD, plant height, number of leaves, leaf unfolding rate, flowering date, and number of shoots (Langton 1981; Fehr 1987; Anderson and Ascher 2004). Such h^2 estimates are a ratio of the total genotypic (additive, dominance, and epistasis) to the phenotypic variances. Confidence intervals for each h^2 estimate were calculated (Knapp *et al.* 1985). Since a random group of cultivars was chosen from commercial products, the reference population is genotypes of the species, rather than a segregating population (Fehr 1987).

Experiment No. 2. Garden Performance – Rerflowering, Frost tolerance, Winter hardiness, Performance the following year

After terminating Experiment No. 1, mature *L. longiflorum* 'Nellie White' and *L. xformolongi* F₁ hybrid plants were cut back on 15 May 2000, planted in the University Of Minnesota flower breeding test fields (St. Paul Campus, 45°N lat., St. Paul, MN USA) on 15 June 2000, and grown for the 2000-2001 field seasons under natural photoperiod and temperature conditions. The field had a progression of LD to SD from 1 June (15.2 h) to 1 Oct. (11.7 h) (United States Nautical Almanac Office 1977). Mean day/night temperatures during 15 June 2000 – 30 Sept 2000 were 25.6/14.4°C, while 2.8/-6.7°C and 25.6/13.9°C mean temperatures were recorded during the periods of 1 Oct 2000 – 31 May 2001 and 1 June 2001 – 30 Sept 2001, respectively (<http://climate.umn.edu>). Plants had been growing for ~10 months (since the commencement of Exp. 1) when they were transplanted into the field in rows 1 m apart and plants 108 cm O.C. within rows. For ease of tracking and correlating observed responses with the photoperiod treatments in Exp. No. 1, each plant was tagged with a unique genotypic identifier that denoted the species, cultivar, photoperiod treatment, and replication number. In the fall 2000, prior to a freeze, one daughter bulb from each of 10 selected, early-flowering genotypes were removed from the field and potted. These daughter bulbs were kept in the greenhouse (same conditions as Exp. 1) under LD for two years.

Data recorded for Experiment No. 2 during the 2000 field growing season consisted of flowering dates, number of stems/genotype, number of flowers/stem, and frost tolerance (after ≥ 1 frosts during Sept-Oct 2000). The first two frosts in fall 2000

occurred on the 24th and 25th Sept.; frost tolerance data was recorded on 2 Oct. During 2001, winter hardiness was recorded on 1 May 2001, as well as the following during the growing season: date of first rerflowering, numbers of stems/genotype, number of flowers/stem, and frost tolerance. Frost tolerance, i.e. the lack of post-freezing ruptured cells which turn brown when the air temperature exceeds 0°C, was determined by macroscopic, visual inspection with a hand lens of floral and leaf tissues the morning after a frost was recorded. If no cells within a plant were ruptured or discolored then such a genotype was rated as frost tolerant. Parametric data were analyzed as in Exp. 1 using SPSS (University of Chicago, Chicago, IL, USA). Non-parametric statistical tests (Kruskal-Wallis or 1:1 χ^2) for traits which violated ANOVA assumptions (flower bud counts, number of shoots/bulb; frost tolerance, winter survival) were grouped by photoperiods (short days vs. long days) to test for significance. Broad sense heritability (h^2) estimates (entry-mean basis) and confidence intervals (CI) were calculated (see Exp. No. 1) for number of shoots in 2000 and 2001, and number of flowers in 2000 (Langton 1981; Fehr 1987).

RESULTS

Experiment No. 1

Seed germination of the hybrid *L. xformolongi* cultivars ranged from 4% ('Raizan No. 3) to 15% ('Augusta F₁'), while the resultant yield potentials ranged from 3.5 to 12.5%, respectively (Table 1). Three albino seedlings also germinated in the 'Raizan' series. As a result of low germination, only limited numbers of seedlings could be evaluated.

There were no significant photoperiod effects on leaf unfolding rates, plant height, number of leaves, or flowering dates (Table 2). Photoperiod had a significant effect on VBD when all genotypes were included (Table 2), but no significance if 'Nellie White' was removed from the analysis ($F = 1.6, P = 0.2$). Thus, in subsequent analyses the SD and LD treatments were pooled. Cultivars were highly significant ($P \leq 0.001$) for all traits (Table 2). There were no significant cultivar \times photoperiod interactions except for flowering date ($P = 0.04$) (Table 2). Flower bud counts were significant ($P < 0.05$) for 'Augusta F₁' and 'Nellie White' using the Kruskal-Wallis χ^2 test (grouped by photoperiod). The number of shoots/bulb were significant only for 'Sakigake Raizan' ($P < 0.01$), based on Kruskal-Wallis χ^2 tests.

Mean number of days to VBD ranged from 208.1 d ('Sakigake Raizan') to 254.3 d ('Raizan No. 3') (Table 3). This is in contrast to 'Nellie White' which reached VBD in 161.9 d, although these are 2-3 year-old vernalized comparison bulbs rather than seedlings (Table 3). 'Nellie White' flowered significantly earlier than all other cultivars. 'Sakigake Raizan' was significantly earlier for VBD than 'Raizan No. 2' and 'Raizan No. 3' whereas 'Raizan No. 3' was the latest of all cultivars (Table 3). 'Augusta F₁' and 'Raizan No. 1' overlapped in distribution with 'Sakigake Raizan' and 'Raizan No. 2' (Table 3). Broad sense heritability for VBD was $h^2 = 0.93$ with a 95% CI of 0.87 to 0.99.

Leaf unfolding rates were negatively, but not significantly, correlated with the number of days to VBD ($r = -0.13$; Table 4). 'Nellie White' had the significantly fastest mean leaf unfolding rate of 4.5 leaves/week (Table 3) and 'Sakigake Raizan' was the slowest (1.9 leaves/week). There

Table 1 Percent germination and percent yield potential of seed-propagated *Lilium xformolongi* hybrid cultivars used in Exp. No. 1.

Cultivar	Seed lot no.	No. seeds sown (N)	Percent germination	Percent yield potential
Augusta F ₁	9149	200	15.0	12.5
Raizan No. 1	8159	200	12.5	11.5
Raizan No. 2	8148	200	10.5	10.5
Raizan No. 3	8418	200	4.0	3.5
Sakigake Raizan	9097	200	12.0	10.5

Table 2 ANOVAS (F values, P values) for visible bud date, leaf unfolding rate / wk, plant height, total leaf number, and number of days to flowering for five seed-propagated *L. xformolongi* cultivars (Exp. No. 1). ANOVA assumptions are not valid for flower bud counts and the number of shoots / bulb^a.

Factor	Visible bud date	Leaf unfolding rate / wk	Plant height (cm)	Total leaf number	No. of days to flowering
Cultivar	49.1 *** ^b	6.2 *** ^b	33.4 *** ^b	9.8 *** ^b	40.56 *** ^b
Photoperiod	49.1 ***	0.1 ns	0.01 ns	0.73 ns	0.36 ns
Cultivar x photoperiod	1.6 ns	0.7 ns	0.93 ns	1.55 ns	3.7 *

^a The ANOVA assumptions are violated and no transformation could make the variances equal. Instead, refer to the non-parametric statistical tests performed for these two parameters (see text).

^b *, **, ***, ns denote significant P values at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and not significant, respectively.

Table 3 Mean values for plant growth traits (Exp. No. 1; visible bud date, leaf unfolding rate / wk, plant height, total leaf number, flower bud count, number of days to flowering, and number of shoots/bulb) for five F₁ seed-propagated *Lilium xformolongi* cultivars and vegetative (clonal) bulbs of *L. longiflorum* 'Nellie White' pooled for short and long day photoperiods (see text). Mean separations were based on Tukey's 5% H.S.D. test.

Cultivar	Visible bud date	Leaf unfolding rate / wk ^a	Plant height (cm)	Total leaf number	Flower bud count ^b	No. days to flowering	No. shoots / bulb ^b
<i>Lilium xformolongi</i>							
Sakigake Raizan	208.1 b	1.9 a	122.4 bc	58.1 a	3.1	247.1 b	3.8
Augusta F ₁	229.8 bc	3.1 b	164.0 d	103.1 c	6.9	266.1 bc	1.7
Raizan No.1	225.9 bc	2.4 ab	139.8 cd	76.8 ab	3.7	250.0 b	3.6
Raizan No. 2	236.8 cd	2.9 b	143.8 cd	98.3 bc	4.2	276.6 c	2.9
Raizan No. 3	254.3 e	2.8 b	98.5 b	100.2 bc	4.7	305.7 d	1.5
<i>L. longiflorum</i>							
'Nellie White'	161.9 a	4.5 c	62.0 a	97.4 bc	5.2	213.4 a	1.0

^a Leaf unfolding rate per wk = total number of leaves unfolded / number of wks to visible bud date (De Hertogh, 1996).

^b ANOVA and mean separations 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).

Table 4 Correlation (r values) matrix and significance (P values) between visible bud date, leaf unfolding rate / wk, plant height, total leaf number, flower bud count, number of days to flowering, and number of shoots / bulb (Exp. No. 1).

Factor	Visible bud date	Leaf unfolding rate / wk	Plant height (cm)	Total leaf number	Flower bud count	No. of days to flowering	No. of shoots / bulb
Visible bud date	----						
Leaf unfolding rate / wk	0.13 ns ^a	----					
Plant height	0.06 ns	0.02 ns ^a	----				
Leaf no.	0.53 ***	-0.21 ns	0.47 *** ^a	----			
Flower bud count	0.28 *	-0.1 ns	0.47 ***	0.67 *** ^a	----		
No. days to flowering	0.82 ***	-0.12 ns	-0.05 ns	0.47 ***	0.23 ns ^a	----	
No. shoots / bulb	-0.24 *	0.2 ns	-0.2 ns	-0.26 *	-0.29 *	-0.27 * ^a	----

^a *, **, ***, ns denote significant P values at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and not significant, respectively.

were no significant differences between all other cultivars for this trait. Despite displaying the slowest leaf unfolding rate, 'Sakigake Raizan' reached VBD the fastest due to its lowest leaf number (Table 3). While 'Augusta F₁', 'Raizan No. 1', 'Raizan No. 2', and 'Raizan No. 3' had the same leaf unfolding rates, 'Raizan No. 3' differed from these cultivars being the latest to reach VBD. Broad sense heritability for leaf unfolding rate was identical to VBD.

Mean plant height of the *L. xformolongi* seedlings ranged from 98.5 cm ('Raizan No. 3') to 164 cm ('Augusta F₁') (Table 3); both of these cultivars were significantly different, although both overlapped with one or more of the seed-propagated other cultivars. In contrast, 'Nellie White' had an average plant height of 62 cm despite having comparable total leaf numbers to 'Raizan No. 2' and 'Raizan No. 3'. 'Sakigake Raizan', 'Raizan No. 2', and 'Raizan No. 3' had the greatest level of variation for plant height (S.D. = 32-36 cm), whereas 'Augusta F₁' had the lowest variation (S.D. = 18 cm). Despite having the shortest plant height, 'Raizan No. 3' was the last to reach VBD (due to higher leaf no.), the date in which visible flower buds indicate the termination of the main stem's vegetative growth. In contrast, 'Sakigake Raizan', which reached VBD in the second earliest group, overlapped with 'Raizan No. 3' for plant height (Table 3). Such differences in response between plant height and VBD is reflected in the negative but not significant correlation of $r = -0.06$ (Table 4). Plant height had a positive correlation with leaf unfolding rate ($r = 0.02$) although this was also not significant (Table 4). Broad sense heritability for plant height was $h^2 = 0.97$ with a 95% CI of 0.93 to 0.99.

The number of leaves ranged from an average of 58.1 ('Sakigake Raizan') to 103.1 ('Augusta F₁') (Table 3), with all other cultivars being intermediate. 'Sakigake Raizan'

had the lowest number of leaves. 'Nellie White' (97.4 leaves) was not significantly different than 'Raizan No. 3', 'Raizan No. 2', and overlapped with 'Raizan No. 1' and 'Augusta F₁' (Table 3). Leaf numbers were highly significantly correlated with VBD ($r = 0.53$) and plant height ($r = 0.47$, Table 4). Broad sense heritability for leaf number was $h^2 = 0.81$ with a 95% CI of 0.76 to 0.86.

Average flower bud counts were as high as 6.9 ('Augusta F₁'; Fig. 1A) and as low as 3.1 ('Sakigake Raizan') (Table 3) in comparison to 'Nellie White' at 5.2 flowers. 'Augusta F₁' had more flowers than all other seed-propagated cultivars (Table 3; Fig. 1A). 'Nellie White' had the lowest variation in flower bud counts between replications (S.D. = 0.5) in contrast to the seed cultivars (S.D. = 2-3). Flower bud count correlations were highly significant with plant height ($r = 0.47$) and number of leaves ($r = 0.67$; Table 4), but barely significant for VBD.

'Nellie White' flowered the earliest (213.4 d), while the *L. xformolongi* cultivars flowered within 247.1 d ('Sakigake Raizan') and 305.7 d ('Raizan No. 3') (Table 3). As would be expected, 'Nellie White' SD plants flowered ~14 d later than those receiving LD (data not shown). There were no significant differences in flowering dates between 'Raizan No. 1', 'Augusta F₁', and 'Sakigake Raizan' (Table 3). 'Raizan No. 3' was significantly later than all other cultivars. The earliest mean flowering date was 237 d ('Sakigake Raizan', 'Raizan No. 1) under LD when photoperiod treatments were not pooled (data not shown). Nonetheless, given the difference in bulb age for 'Nellie White' (2-3 yrs + 213 d) the seed-propagated cultivars were surprisingly early flowering. Both 'Augusta F₁' and 'Nellie White' flowered in time for Easter in 2000. Again, the range in variation of *L. xformolongi* cultivars (S.D. = 22-32) was greater than that observed for clonal 'Nellie White' (S.D. =



Fig. 1 Flowering of seed-propagated *Lilium xformolongi*. (A) 'Augusta F₁' (plant No. 1, Exp. 1) hybrid grown under long days and displaying a high flower bud count; Buds are 20 cm in length. (B) 'Raizan No. 1' (plant No. 6, Exp. 1) showing the multiple daughter shoots emerging following the flowering of the terminal stem. Bar (pot label): 15 cm. Photo credit: Neil Anderson.



Fig. 2 Flowering of day-neutral, reflowering, frost-tolerant, seed-propagated *Lilium xformolongi* 'Raizan No. 1' (long day photoperiod, plant No. 6 from Exp. 1) in the field for Exp. 2 after several frosts ($\leq 0^{\circ}\text{C}$) had occurred. Field stakes demarcate each individual plant with multiple stems at all stages of development from juvenile (non-flowering) to post-anthesis (seed pods). Lily seedlings in this photograph were sown in July, 1999, and had flowered in Exp. No. 1 (see text). Note: Bar (field stakes): 30 cm. Photo credit: Neil Anderson; taken on 30 Oct. 2000.

7.1). As would be expected, the number of days to flowering was highly correlated with VBD ($r = 0.82$; **Table 4**) as well as leaf number ($r = 0.47$); all other correlations were not significant. Broad sense heritability for flowering date was $h^2 = 0.91$ with a 95% CI of 0.90 to 0.92.

Mean number of shoots/bulb ranged from one ('Nellie White') to 3.8 ('Sakigake Raizan', **Table 3**). 'Sakigake Raizan' had a significant Kruskal-Wallis χ^2 . All seed-propagated lilies had > 1 shoot/bulb (**Fig. 1B**). The biological phenomenon of reflowering without vernalization is evident in all seed-propagated cultivars but not for 'Nellie White'. By 10 Dec 1999 (154 d after seed germination; 42 d after 'Nellie White' bulbs were received), with the exception of 'Raizan No. 3', all seed-propagated cultivars had genotypes with the second shoot emerging even though the primary shoot had not yet reached VBD. Both 'Sakigake Raizan' and 'Raizan No. 1' (**Fig. 1B**) had the highest mean number of shoots (**Table 3**). Shoot number was negatively, but significantly, correlated with all traits except for leaf unfolding rates and plant height (**Table 4**). Each shoot developed a bulb at the base, termed a 'daughter bulb' (Dole and Wilkins 2005).

Experiment No. 2

Reflowering of the lilies in the field during 2000 occurred as early as 1 July ('Augusta F₁', SD, **Table 5**) – a mere 42 d period following cut back on 15 May – and as late as 5 August ('Raizan No. 2', LD) with flowering continuing through the growing season as additional flower stems were produced (**Fig. 2**). This is in contrast to Exp. No. 1 where 'Augusta F₁' was not the earliest to flower (**Table 4**). The mean number of flowering stems/bulb in 2000 ranged from 1.3 ('Nellie White', SD and LD; 'Raizan No. 3', LD; **Table 5**) to 4.1 ('Raizan No. 1', LD). The daughter bulbs dug in the fall 2000 and grown in the greenhouse flowered continuously under LD for ~ 2 yrs until they succumbed to lily symptomless virus (LSV; Linderman *et al.* 1976).

Flower stem numbers in 2000 and 2001 were not significantly correlated ($r = -0.003$). Kruskal-Wallis χ^2 tests for the number of flower stems were significant only for 'Raizan No. 1'. In 2001, the range of average number of flowers stems/bulb exceeded that of 2000: 1.0 ('Augusta F₁', **Table 5**) to 9.9 ('Sakigake Raizan', SD), although only 'Sakigake Raizan' was significant ($P < 0.001$) for the Kruskal-Wallis χ^2 test. The genetic component remains a significant factor but photoperiod (Exp. No. 1), had no residual effect on reflowering shoots in the field during 2000 and 2001. 'Nellie White' exhibited multiple reflowering stems, in contrast with Expt. No. 1 (**Table 4**) since multiple daughter bulbs were initiated, but not removed from the mother bulb prior to field planting. Broad sense heritability for number of stems in 2000 was $h^2 = 0.78$ with a 95% confidence interval of 0.74 to 0.82 and in 2001, $h^2 = 0.36$ (95% CI = 0.0).

Flower numbers/stem in 2000 ranged from an average of 1.3 ('Raizan No. 1', SD; 'Raizan No. 2', LD; **Table 5**) to 4.0 ('Nellie White', SD). Broad sense heritability for number of flowers was $h^2 = 0.74$ with a 95% CI of 0.71 to 0.77. Kruskal-Wallis χ^2 tests were not significant ($P > 0.05$) for the number of flowers/stem in 2000 and 2001. Flower number was significantly correlated with % frost tolerance in 2000 ($r = -0.309$, $P < 0.05$) and 2001 ($r = 0.445$, $P < 0.05$), but not any other traits.

Frost tolerance was an unexpected trait (**Fig. 2**). Since lilies are not normally in flower during the fall season in northern latitudes, frost tolerance would rarely be observed. The *L. xformolongi* hybrids segregated for frost tolerance, ranging from 25% ('Sakigake Raizan', SD) to 75% ('Augusta F₁', **Table 5**). 'Nellie White' could not be evaluated for frost tolerance since it flowered only in late July (**Table 5**), did not possess reflowering capabilities, and, thus, was not in flower during the frost period. Photoperiod effect on frost tolerance was significant for 'Augusta F₁' (1:1 $\chi^2 = 8.6$, $P < 0.01$). All other cultivars did not differ significantly for frost tolerance response between photoperiods.

During the 2000-2001 winter period, there were 90 occurrences of two consecutive diurnal periods in which the minimum ambient air temperature was from $\sim -10^{\circ}\text{C}$ to $\sim +5^{\circ}\text{C}$ (<http://climate.umn.edu/>). The lowest temperature recorded during this winter period was -26.1°C on 25 Dec. 2000. *Lilium xformolongi* winter survival ranged from a mean of 0.0% ('Augusta F₁', LD; 'Raizan No. 3', SD and LD, **Table 5**) to 87.5% ('Sakigake Raizan', SD). *L. longiflorum* 'Nellie White' was intermediate with 33.3% (SD) and 37.5% (LD). 'Raizan No. 1' (20%) and 'Raizan No. 2' (19%) had the most similar % winter survival, whereas 'Raizan No. 3' had 0% winter survival. *L. longiflorum* 'Nellie White' had 35% winter survival (**Table 5**). There were significant differences in winter survival for photoperiods in 'Augusta F₁' (1:1 $\chi^2 = 12.5$, $P < 0.001$) and 'Raizan No. 2' (1:1 $\chi^2 = 4.2$, $P < 0.05$). Winter-hardiness was not significantly correlated with frost tolerance ($r = 0.13$) nor any other traits (data not shown).

Table 5 Mean garden performance data (Exp. No. 2) for the 2000-2001 field seasons in St. Paul, MN USA (45°N Lat.) for five seed-propagated *L. xformolongi* hybrids and vegetative (bulbs) *Lilium longiflorum* 'Nellie White' (regrown from Expt. No. 1 photoperiod treatments: SD—short days; LD—long days; n = 8 plants / trmt): 2000 and 2001 seasons—reflowering date, number of flower stems, number of flowers/stems; 2000 season only—% frost tolerance (+); 2001 season only—percent winter survival.

Species & cultivar(s)	Expt. No. 1 photoperiod treatment	2000					2001		
		Reflowering date	No. of flower stems	No. flws / stem	% Frost tol. (+)	% Winter survival	Reflowering date	No. of flower stems	No. flws. / stem
<i>L. xformolongi</i>									
'Sakigake	SD	15 July	3.2	1.6	25.0	87.5	8 June	9.9	1.9
'Raizan'	LD	5 July	2.8	2.0	38.0	71.4	1 June	2.8	2.6
'Augusta F ₁ '	SD	1 July	2.7	2.3	75.0	25.0	17 Aug	1.0	7.0
	LD	1 Aug	2.3	2.7	43.0	0.0	-- ^c	-- ^c	-- ^c
'Raizan No. 1'	SD	--- ^a	2.7	1.3	30.0	25.0	17 Aug	3.0	1.5
	LD	21 July	4.1	2.4	30.0	14.2	10 July	2.7	1.3
'Raizan No. 2'	SD	21 July	3.7	2.2	50.0	12.5	10 July	3.0	1.0
	LD	5 Aug	3.0	1.3	38.0	25.0	30 June	-- ^d	-- ^d
'Raizan No. 3'	SD	--- ^a	1.4	1.7	50.0	0.0	-- ^c	-- ^c	-- ^c
		--- ^a	1.3	2.0	50.0	0.0	-- ^c	-- ^c	-- ^c
<i>L. longiflorum</i>									
'Nellie White'	SD	21 July	1.3	4.0	-- ^b	33.3	7 July	2.7	3.0
	LD	15 July	1.3	3.8	-- ^b	37.5	14 July	2.7	3.0

^a In bud when the first frost was recorded.

^b Not in flower during the frost period.

^c Dead.

^d Flower stems were stolen from the fields before the data could be collected

DISCUSSION

While seed germination experiments were conducted within the recommended temperature range of 15-20°C (Dai-Ichii Seed Co. 1999; Sakata Seed Co. 1998-2000), % seed germination was variable between *L. xformolongi* hybrids (Table 1). Shii (1983) recommended 15°C as the optimal germination temperature for both *L. longiflorum* and *L. formosanum* seeds, although both are sensitive to high temperatures (30/25°C). Roh and Sim (1996) recommended a slightly lower temperature (14°C) for *L. xformolongi* 'Snow Line' germination, however, following a 1-wk. 29°C pre-treatment. Nonetheless, similar ranges in germination to those found in Exp. No. 1 (Table 1) have been recorded for *L. formosanum* (Shii 1983; Carpenter and Ostmark 1991; Mynett 1997) and *L. xformolongi* (Watanabe 1993; Roh and Sim 1996; Mynett 1997). Future research is necessary to elucidate specific germination temperature requirements for *L. xformolongi* hybrid cultivars and whether secondary dormancy (ecodormancy) exists to resolve these discrepancies.

The low seed germination for all *L. xformolongi* cultivars in Exp. No. 1 (which were sown during the summer; Table 1) may have been caused by high temperature-induced secondary dormancy. Such a phenomenon was reported to exist in *L. xformolongi* 'Snow Line' at >20°C temperature during germination (Roh and Sim 1996). While the *L. xformolongi* commercial cultivars used in Exp. No. 1 did not include 'Snow Line' (not available), the 'Raizan' series, 'Augusta', and 'Sakigake Raizan' hybrids may be genetically related and/or share high temperature-induced secondary dormancy. Breeding and selection against this trait would be required to increase seed germination to commercially acceptable levels (PanAmerican Seed Co. 2005).

Yield potential differences existed between *L. xformolongi* cultivar seed lots (Table 1). Few *L. xformolongi* hybrids had % yield potential values within the range for commercial seed-propagated crops (PanAmerican Seed Co. 2005). Similar findings were reported by Roh and Sim (1996) where 66% was the highest yield potential (no. of uniform seedlings) found when 'Snow Line' was germinated at 17°C. At higher germination temperatures (17-26°C), yield potential ranged from 0% to 36% (Roh and Sim 1996). This demonstrates that considerable genetic and environmental variation exists for yield potential within *L. xformolongi* cultivars, as well as the demonstrable need for directed selection and improvement in flower breeding programs (Anderson 2004a). Selection for increased stability of this trait across temperature ranges would be

required to improve yield potential in plug production.

Genetic variation in *L. xformolongi* cultivars existed for all other morphological traits (Table 3). For instance, plant height ranged from 98.5 to 164 cm (Table 3), depending on the cultivar. Cultivar-dependent variability has been noted previously (Ho *et al.* 2006) and was also present in wild parental populations, e.g. *L. longiflorum* var. *formosanum* ranges in plant height from 45.8 cm to 148.7 cm (Wen and Hsiao 2001). 'Sakigake Raizan', 'Raizan No. 2', and 'Raizan No. 3' had the greatest level of variation for plant height (S.D. = 32-36 cm), whereas 'Augusta F₁' had the lowest variation (S.D. = 18 cm). The genetic variation for all traits suggests that further inbreeding is required, particularly if such hybrids were to be used for pot plant production where plant height variation is more noticeable than in cut flowers. Fortunately, most *L. xformolongi* are self-compatible (Anderson and Dunn 2003) which will allow for trait selection and increasing homozygosity.

Vernalized (CC) *L. longiflorum* bulbs flowered in both photoperiod treatments under greenhouse conditions (Table 3). In the case of *L. longiflorum* 'Nellie White' SD-treated bulbs required several days or weeks longer than LD to reach VBD and flowering, thus reconfirming the quantitative LD response for flower bud initiation with temperatures ≤ 21°C (Roh and Wilkins 1973; Lange and Heins 1988). Providing LD after vernalization, when Easter is early (Miller 1992), causes increased plant height and reduced flower bud counts in *L. longiflorum* (Smith and Langhans 1962). Following the LD treatment, leaf unfolding rates are closely monitored and manipulated with temperature to ensure VBD is reached with precision (Karlsson *et al.* 1988).

The rapid flower bud initiation (mean VBD in 208-254 d from sowing; Table 3) and development (flowering in 247-306 d, Table 3) of seed-propagated *L. xformolongi* hybrids without vernalization is unprecedented (Fig. 2), indicating a short juvenility period (Fukai *et al.* 2005; Rhee *et al.* 2005). Previous reports of flowering seed-propagated *L. xformolongi* were nine months or ~270 d from sowing with a vernalization treatment in an "unheated greenhouse" (Mynett 1997), a significantly longer duration of time than most of the hybrids tested in the current experiments. Other research did not quantify flowering dates (Shii 1983). The only hybrids to exceed the nine month duration for flowering were 'Raizan No. 3' and 'Raizan No. 2' (Exp. No. 1; Table 3). Interestingly, comparisons of 'Augusta F₁' seedling (Exp. No. 1; Table 3) performance with scale-bulblet-derived plants (*cf.* Tables 1-3, Goo and Kim 2008) show

that seedlings (164 cm, **Table 3**) have greater plant height than scale-bulb-derived plants (26.3-104 cm; Goo and Kim 2008). Other plant traits showed were similar: leaf number (103.1, **Table 3** vs. 7.2-40.5), flower number (6.9, **Table 3** vs. 1.1-1.3), and number of days to flowering (266, **Table 3** vs. 11.8-121.3). The lack of stability in time to VBD and flowering, as evidenced by the hybrid seed lots used (**Table 3**) indicates genotype \times environment (G \times E) interactions. Stability would be a requirement to ensure programmable commercial production of seed-propagated hybrids as flowering potted plants for Easter sales or cut flowers year-round (Anderson 2000, 2003).

Contrary to *L. longiflorum* 'Nellie White' which is sensitive to photoperiods and high temperatures during flower bud initiation (Wilkins 1980), the ability of *L. xformolongi* to initiate and develop flowers at $>21^{\circ}\text{C}$ regardless of photoperiod (day neutrality) indicates different gene(s) and genetic variation which could be exploited by lily breeding programs (Griffiths 1934; Anderson and Dunn 2003). Zlesak and Anderson (2010) recently demonstrated *L. formosanum*, one of the hybrids' parents, is the source for these flowering traits. The discovery of day-neutral, seed-propagated *L. xformolongi* is novel, e.g. 'Raizan No. 1' (Seed Lot #8159) VBD and flowering dates were nearly equivalent when grown under SD and LD in the greenhouse. This cultivar would be an excellent source for day neutrality with the inclusion of SD and LD photoperiods to select for this trait as developed for other floriculture crops (Anderson and Ascher 2001; Erwin and Warner 2002; Anderson and Ascher 2004).

Previous reports of day neutral species (flowering both in SD and LD) with equivalent leaf numbers under both photoperiods (Anderson and Ascher 2001; Erwin and Warner 2002; Anderson and Ascher 2004) were similar for most, but not all, seed-propagated *L. xformolongi*. For instance, all *L. xformolongi* cultivars in Exp. No. 1 (**Table 3**) were day-neutral since photoperiod was not significant for any trait recorded (**Table 2**; **Fig. 2**), despite mean variable leaf numbers. Day neutrality in *L. xformolongi* seedlings may be unique, since clones derived from scale-leaves and scale-bulbs of 'Augusta F₁' (Goo and Kim 2008) had $<50\%$ flowering under different photoperiods and temperatures. Transference of day neutrality to other lily species and cultivars also requires selection, since interspecific hybrids between *L. xformolongi* \times Asiatic and Oriental lilies are day length sensitive and not day-neutral (Rhee *et al.* 2005) or require vernalization (Horita *et al.* 2003; Fukai *et al.* 2005).

Reflowering of seed-propagated *L. xformolongi* occurred in the field (Exp. No. 2, **Table 5**; **Fig. 2**) environment, similar to that noted for wild populations of *L. formosanum* (Shii 1983; Walters 1983). Reflowering took significantly less time than the initial (first) flowering, as quickly as 42 d for 'Augusta F₁' (**Table 5**). It should be noted that reflowering of the second shoot (**Table 5**) did not follow the same flowering sequence across cultivars noted for the first shoot (**Table 3**). The continuous reflowering of the daughter bulbs (dug from the field in 2000, Exp. No. 2) for 2 yrs is also novel and unexpected since previous reports had never tested genotypes for this trait (Watanabe 1993; Roh and Sim 1996; Mynett 1997; Rhee *et al.* 2005; Sato *et al.* 2010). From these experiments, significant and useful genetic variation exists for the reflowering trait. Commercial production of seed-propagated cut flower *L. xformolongi* has followed the recommendation used for bulb-propagated cut lilies (De Hertogh 1996; Dole and Wilkins 2005), i.e. 1x flowering and harvest followed by bulb disposal (Watanabe 1993; Mynett 1997; Sakata Seed Co. 1998-2000; Dai-Ichii Seed Co. 1999; Sato *et al.* 2010). In fact, several research groups have focused on propagating and forcing daughter bulbs from scale-leaves and scale-bulbs (Ishimori *et al.* 2007; Goo and Kim 2008) to extend the flowering/harvest season of cut flower *L. xformolongi*. Vegetative propagation of *L. xformolongi*, however, resulted in variability for cooling (vernalization-requiring), sprouting, bolting and flowering which were absent in the original seedlings (Goo

and Kim 2008). Ishimori *et al.* (2007) found this was due to the need for low temperatures plus cytokinins (6-benzyladenine). Our research demonstrates that gene(s) for reflowering exist in several cultivars (**Table 5**) and would be easier to exploit without these clonally-induced variations. However, the genetic potential and G \times E variation for this trait is unknown and would require extensive breeding and selection.

Previous research has documented the rare occurrence of reflowering lily cultivars, although this is restricted to 2X/year rather than continuous reflowering and occurs in vegetatively-propagated bulbs which received their required vernalization treatment prior to flowering, e.g. 'Lorina' (www.bloom.co.nz), 'Prima' (Mynett 1992), and 'Pirate' (P. Ascher 2000, pers. comm.). Only the research on 'Prima' has been conducted scientifically; the other cultivars are based on untested observations. 'Prima' only reflowered if the bulbs were lifted from the field in August, vernalized for 6-8 wks., and then forced for December flowering (Mynett 1992). Clearly the reflowering trait of *L. xformolongi* could be implemented in commercial cut lily production in the field or greenhouse for multiple harvests (Anderson 2003; Zlesak and Anderson 2010). Likewise, reflowering could be used in potted plant production and as garden perennials (Anderson 2003).

The question arises whether the reflowering trait is similar to summer sprouting reported in *L. longiflorum*. Summer sprouting is defined as the growth of a daughter bulb without vernalization (Van Tuyl 1985). Genetic (genotype) and temperature are factors implicated in summer sprouting (Van Tuyl 1985). Field Easter lily bulb producers, propagators, and breeders select against this trait since such bulbs rarely can be programmed to flower for Easter (Roberts 1975), although the phenomenon continues to resurface each year in production fields (H. Harms and L. Riddle 2002, pers. comm.). Traits such as flowering without vernalization, day neutrality, and reflowering may be related to summer sprouting or an independent biochemical pathway.

Reflowering, seed-propagated *L. xformolongi* hybrids continuously initiated daughter bulbs in the reflowering experiments in the field (**Table 5**; **Fig. 2**) or for as long as 2+ yrs (as in the case of daughter bulbs dug from the field in fall 2000 and forced continuously in the greenhouse without vernalization). This unusual phenomenon was not fully realized until late Oct. 2000, when the lilies had multiple daughter shoots in all stages of juvenility to reproductive maturity (**Fig. 2**).

Leaf number was significantly and positively correlated with the number of days to VBD in Exp. No. 1 ($r = 0.53$, $P \leq 0.001$; **Table 4**), similar to previous reports for *L. longiflorum* (Dole and Wilkins 2005). 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 (**Table 4**). A positive relationship between leaf number and VBD as well as flowering occurs in *L. longiflorum* (De Hertogh 1996).

Since flowering in *Lilium* species and hybrids is restricted to a specific time period during the summer growing season (De Hertogh and Le Nard 1993; Dole and Wilkins 2005), plants are normally ripening seed and senescing during the fall when frosts and freezes normally occur. For instance, *L. longiflorum* 'Nellie White' was not flowering during the fall 2000, and could not be screened for floral frost tolerance (**Table 5**). Thus, in the continuously reflowering, seed-propagated *L. xformolongi* (which did not require vernalization) the first-ever opportunity to screen flowers for frost tolerance was unexpected (Anderson 2003). Frost tolerance segregated in the hybrid populations, ranging in expression from 25 to 75% of the hybrid *L. xformolongi* cultivars in Exp. No. 2 (**Table 5**). Photoperiod treatment effects did not affect frost tolerance expression in all cultivars with the exception of 'Augusta F₁' ($1:1 \chi^2 = 8.6$, P

< 0.001). Whether this affect with 'Augusta F₁' is real or due to small sample size is unknown and warrants further investigation. A weak and non-significant correlation was found between frost tolerance and winter hardiness ($r = 0.13$). Frost tolerance could be a trait with market value for field-grown cut lily producers in northern latitudes, allowing for an extension to the growing season after the first and successive frost(s) until a killing freeze (Anderson 2003). Likewise, home gardeners could benefit from such a season extension in lily flowering (Anderson 2003).

Winter hardiness (cold tolerance) is a valuable trait for woody and herbaceous perennials in northern climates (Anderson 2004b; Anderson and Gesick 2004). Since the 2000 winter had adequate snow cover and at least one cold night (-26.1°C) on 25 Dec., winter survival measurements had merit for 45°N lat. (Anderson and Gesick 2004). Winter survival segregated within and between *L. xformolongi* and *L. longiflorum* (Table 5). Some *L. xformolongi* cultivars were not hardy ('Augusta F₁', 'Raizan No. 3', Table 5) while others were more cold tolerant ('Sakigake Raizan', Table 5). The subsequent winter (2001) in the field, which lacked adequate snow cover when severe below zero (< -20°C) temperatures occurred (<http://climate.umn.edu>), resulted in 100% winter kill of all remaining *L. xformolongi* and *L. longiflorum* genotypes which had survived the 2000 winter (data not shown). Thus, bulb survival (winter hardiness) in the field necessitates adequate snow cover when low temperatures occur. Nonetheless, since many lily species and hybrids are winter hardy, gene(s) for winter survival exist in *L. xformolongi* and could be studied for heritability to select more cold-tolerant seed-propagated lilies.

Results from these experiments surpass Dole and Wilkins (1996) objective for day-neutral, non-vernalization requiring Easter lilies. Since it was possible to force at least one seed-propagated *L. xformolongi* cultivar into flowering for Easter (Exp. No. 1) as a potted plant crop, could a breeding program select among the hybrids and backcrosses for potential parents to accomplish such a breeding objective? In Exp. No. 1, 'Augusta F₁' had the greatest uniformity for all traits (lowest S.D.) and had a higher flower bud count than 'Nellie White' (Table 3). 'Raizan No. 1' and 'Raizan No. 3' were not significantly different from 'Nellie White' for leaf unfolding rates/wk in Exp. No. 1 (Table 3). 'Raizan' series, Nos. 1-3, were likewise not significantly different from or overlapped with 'Nellie White' for leaf number (Table 3). Leaf number was highly heritable ($h^2 = 0.81$).

It is unknown whether seed-propagated *L. xformolongi* could be reliably scheduled for Easter lily production, since the date varies each year, given the lack of stability across environments in the current experiments. A breeding program could select for greater stability for all critical traits, however. Should stability not be attainable, seed-propagated *L. xformolongi* could still be used for flowering potted plant and cut flower production for floral holidays with longer sales periods. Nonetheless, seed-propagated trumpet lilies offer several advantages over vegetative Easter lilies, i.e. lack of viruses (which did not always confer plant heights taller than 'Nellie White'), shorter generation time to flower (<1 year vs. 2-3 years for 'Nellie White'), less expensive shipping costs of propagules, and quicker response to increased consumer demands. A proposed crop ideotype has been proposed by Anderson (2003) for *L. xformolongi* in a utility patent filed with the U.S. Patent Office which incorporates the significant traits in the present experiments. Integration of the novel traits in seed-propagated *L. xformolongi* discovered herein would also be valuable additions to potted and cut flower Asiatic and Oriental lilies. The breeding procedures for trait transfer from *L. xformolongi* into colored lilies have already commenced (Fukai *et al.* 2005; Rhee *et al.* 2005; Anderson *et al.* 2009, 2010). Incorporation of some or all of the traits reported here will continue the supply of novel *Lilium* products worldwide.

CONCLUSION

The ability of *L. xformolongi* hybrids to flower in <1 year from sowing without vernalization or photoperiodic compensation for inadequate cooling are unique traits. These traits, coupled together with day neutrality, frost tolerance, winter hardiness and continuous flowering provide this crop with new commercial applications. However, several factors inherent within these hybrids require further breeding and improvement before they could be reliably programmed in production. Significant genotype x environmental variation throughout the entire life cycle exists within *L. xformolongi* hybrids which would complicate flowering this crop for specific weeks or variable target holidays, such as Easter. Improvements in seed germination and yield potential would be critical first steps to ensure integration into plug production, followed by uniform leaf numbers within hybrids, programmability by temperature rather than photoperiod to ensure uniform leaf unfolding rates and visible bud dates and, lastly, high flower bud counts. Since *L. xformolongi* are highly self-compatible, the generation of inbred parents is proposed as a means of concentrating favorable alleles for each of these traits to produce more uniform seed-propagated hybrids.

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