Fragrance Removal in *Lilium* L. Subdivision Orientalis (Oriental lily)

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EXECUTIVE SUMMARY

The Oriental lily is a popular flower. Some enjoy the strong fragrance that accompanies it and others have difficulties with the scent. To reach to market of people who do not like or cannot tolerate the fragrance a new scent-free lily could be designed. Current production practices are reviewed along with potential future practices and an in-depth history of the Oriental lily itself.

I. INTRODUCTION

A. Study Species.

"Lilies are dangerous. They are addictive. Any advocacy of their beauty or interest ought to be accompanied by the equivalent of a government health warning."

Michael Jefferson-Brown

FIGURE 1. Oriental lilies in a variety of colors (http://www.cherrygal.com/cherrygalheirloombulbsorientalliliesfragrantmix-p-14505.html).

Many have fallen under the spell of Oriental lilies, *Lilium* subdivision Orientalis, including myself. Offered in an artist's palette of colors and possess beauty and grace (Figure 1). Oriental lilies also have a highly fragrant perfume that may be overpowering to some people. The complete elimination of their fragrance (volatile compounds) is the goal of this paper. This would make Oriental lilies accessible to those who cannot tolerate their scent but want to enjoy their majesty.

B. Taxonomic Classification and Geographic Distribution in the Wild.

Lilium spp. subdivision Orientalis, Oriental lily, is in the genus *Lilium* and belongs to the family Liliaceae (McRae, 1998). The Oriental lily hybrid has broad, alternate leaves and is late-flowering (McRae, 1998). Usually large and showy (15.2 to 25.4 centimeters) flowers that are bowl-shaped, flat, or reflexed. The fragrance is usually strong and sweet. The lily possesses hypogeal (underground germination), delayed germination (except for a form of *L. brownii* and, rarely, *L. speciosum*). The leaves are scattered with a distinct leaf stalk, the bulb scales are entire and erect, white (except some forms of *L. speciosum*). They

have an erect stem with roots (Jefferson-Brown, 1988.). Lilies stand at 61 – 91.4 cm tall, come in red, purple, pink, white and bicolor and make great cut flowers (Different, 2014). All lily plants survive over multiple years, annually sending up a new stem from the bulb, their underground storage organ. Each stalk contains one or several flowers and they may be poised at various angles. A common pattern of the Liliaceae and other monocotyledonous plants is that the parts of the flower come in sets of three or six (McRae, 1998).

The horticultural classification of lilies includes nine divisions within the genus *Lilium* which is based upon the number of blooms per stem and the shape and posture, or presentation, of the individual flowers (Jefferson-Brown, 1965).

Division I: The Asiatic Hybrids

Division II: The Martagon Hybrids

Division III: The Candidum Hybrids

Division IV: The American Hybrids

Division V: The Longiflorum Hybrids

Division VI: The Trumpet Hybrids

Division VII: The Oriental Hybrids

VII consists of cultivars from a wide range of species and hybrid groups and is arranged into four subdivisions:

VIIA – Trumpet-shaped flowers.

VIIB – Bowl-shaped flowers on 88.9- to -121.92 centimeters, often stem-rooting plants.

VIIC – Recurved flowers on 88.9- to -121.92 centimeters stem-rooting plants.

VIID – Floral umbel contains 12 to 30, fragrant, 20.32- to -30.48 diameter flowers with recurved petals but a flat face.

Division VII contains the Oriental hybrids. This division contains hybrid cultivars and hybrids of *L. auratum, L. speciosum, L. japonicum, L. rubellum*, and to include any of those crossed with *L. henryii.* Oriental lilies are derived from the two "super-lilies" native to Japan, *L. auratum and L. speciosum* (de Graaff and Hyams, 1967). *Lilium auratum*, the Golden-rayed lily of Japan, is fairly wide-ranging in its native land, being found wild from south-western Hokkaido, at latitude of about 36° N., to Honshu, at about 42° N. The species *L. auratum* is excessively susceptible to viruses and virus-free bulbs are difficult to buy. Soil drainage is of most importance, as in the wild they are found growing on steep hillsides, in a mixture of volcanic ash and gravel. The plant responds to overfeeding with swift and sappy growth and then perishing after one good flowering. *Lilium auratum* is very intolerant of lime and salt (de Graff and Hyams, 1967). *Lilium speciosum* comes from further south in Japan, from Shikoku and Kyushu. It is not as winter hardy as *L. auratum*. In the wild and hot-house gardens the glossy green stem may reach 2.1336 meters. The flowers in a fine specimen can be 15.24 centimeters in diameter, but are usually 10.16 centimeters across. The centers are a deep crimson, fading a flush of crimson to white at the margins. The large anthers are sometimes red but usually chocolate –brown.

Division VIII: Contains all hybrids not provided for in any previous division.

Division IX: Contains all true species and their botanical forms.

Two species of lilies are invasive. They are *L. formosanum* and *L.* x*formolongi*, which are invasive in South Africa (Henderson, 2001) and the west coast of North America (Anderson, 2015). In Anatolia, the Madonna lilies, *L. candidum,* were cultivated for a healing salve prepared from its bulbs 1,500 years before the birth of Christ. Ancient Egyptians grew its bulbs for eating and early Greeks and Romans cultivated it for medicine and garden adornment. In the herbal of Dioscorides it is recognizably pictured and described as having healing qualities. At least 1,000 years ago, Chinese, Koreans, and Japanese were growing bulbs of the tiger lily, *L. tigrinum,* for food (Grieve, 1995).

Lilies are very popular garden plants though the major use of lilies in the world is as cut flowers and to a much lesser degree as potted plants. Dozens of new cultivars are introduced each year from breeding efforts in Holland. Nearly all of these cultivars are used in the world cut flower industry, and are bred especially for characteristics as upright facing flowers, rapid and uniform growth and general desirability as cut flower (Different, 2015). In addition, vigor, disease-resistance, and tolerant to virus are qualities that must be present when choosing breeding parents. (McRae, 1998).

II. CROP HISTORY

A. Breeding & Domestication.

In the last 50 years, the lily is grown worldwide one of the most significant flower bulbs and cut flowers. There are over 5000 hectares of bulb production predominately grown in The Netherlands with thousands of cultivars classified in different hybrid groups. Japan and the US performed the first crosses within the section *Sinomartagon*. This was done within the first decade of the 20th century. The species included were *Lilium maculatum*, *L. davidii, L. dauricum, L. bulbiferum* and *L. tigrinum* (de Graaff, 1970). Several hybrid groups were developed, such as the Preston hybrids, the Mid-Century hybrids, the Patterson hybrids, the Harlequin hybrids, etc. (de Graaff, 1970; Rockwell et al., 1961; McRae, 1998). Mid-Century Hybrid 'Enchantment' bred by Jan de Graaff in 1944 was a turning point. In 1977, there were over 700 hectares of lilies grown in The Netherlands; this area consisted of the first polyploid (triploid and tetraploid) lily introductions. Currently the majority of cultivars grown are polyploid (Table 1).

Dutch breeding programs began after World War II. In 1970, the importance of the lily crop was discovered. Asiatic hybrids, which include early flowering lilies with upright flowers, single or in an umbel, those with outward –facing flowers, or those with pendent flowers (Jefferson-Brown, 1995). Asiatic hybrids were the mainstay for 30 plus years (1970-2000). During this time Oriental hybrids, originating from crosses of predominately *L. auratum* and *L. speciosum* from the *Archelirion* section,

evolved. Lilies with huge, striking, mostly pink and white flowers were introduced. They became the most important hybrid group in the 1990s. From this group the earliest up-facing Oriental hybrid, bred by Minnesota breeder Leslie Woodriff (introduced in 1975) was the chief cultivar for over 25 years. Today, Oriental hybrids are the eminent group of lilies.

At the onset of pollination, cut styles, embryo rescue and polyploidization techniques were used (Asan, 1978, 1980; Asano and Myodo, 1977a,b: Barba-Gonzales, 2005; Barba-Gonzalez et al., 2004, 2005, 2006; Lim, 2000; Lim and Van Tuyl, 2004, 2006; Lim et al., 2001, 2004, 2007; Van Tuyl and Boon, 1997; Van Tuyl et al., 1989, 1991, 1992, 2002, 2003) to produce an assortment of intersectional hybrids. The starting assembly of intersectional hybrids, the *Longiflorum* x Asiatic hybrids or LA hybrids, was created 20 years after the Oriental hybrids, by interspecific hybridization between hybrids of different sections, the *Longiflorum* and the Asiatic group. They are triploid, since these are backcrosses from a chromosome doubled F_1 LA hybrid with an Asiatic hybrid. This group has become more essential than the Asiatic hybrid group and basically replacing them in the industry.

The breeding of other intersectional crosses, primarily triploid hybrids (having superior growth vigor and plant habit) like LO (*Longiflorum* x Oriental), OT (Oriental x Trumpet) and OA (Oriental x Asiatic hybrids) began. Since the LA-hybrids largely took the place of the Asiatic hybrids and the LO-hybrids the *Longiflorums*, it can be predicted that Orientals are partially going to the way of the OTs.

In addition to the creation of the seven main hybrid groups (O, A, L, T, LA, LO and OT), other successful breeding of species occurred as seen in Figure 1. Species not integrated into the commercial assortment consist of *L. candidum* (section *Lilium*) crosses with *L. henryii* and *with L. longiflorum*. Using *Longiflorum* as the female parent more crosses created are *L. auratum, L. bakerianum, L. canadense, L. concolor, L. dauricum, L. henryii, L. kelloggii, L. lankongense, L. lophophorum, L. monadelpum, L. martagon, L. hansonii, L. nepalense, L. pardinum. L. pumilum* and *L. semper vivoideum* as the male parent. With Oriental as the female parent, crosses were made with *L. dauricum, L. nepalense* and *L. pardalinum*. Look forward to the addition of some of these crosses in the assortment.

TABLE 1. The top 25 most widely grown lily cultivars in The Netherlands in 2010 (provided by The Dutch Flower Bulb Inspection Service). Given is the hybrid group (A=Asiatic, O=Oriental, L=Longiflorum, LA, LO and OT), the ploidy level (2=diploid, 3=triploid, 4=tetraploid, the bulb production area (in ha) grown in The Netherlands in 2010 and 1994, the Breeder (VH=Vletter and Den Haan BV, (recently joined with Marklily, M=Makbreeding, IM=Imanse, RVZ=Royal van Zanten, WB=World Breeding) and the year of introduction (Intro).

			Production area (ha)			
Cultivar	Group	Ploidy Level	2010	1994	Breeder	Intro.
Sorbonne	O	Diploid	200		VH	1994
Siberia	O	2	193	6	Mak	1992
Robina	OT	3	84		Marklily	2004
Tiber	O	2	71		VH	1994
Conca dór	OT	3	67		VH	2002
Brindisi	LA	3	67		VH	2002
Rialto	O	2	63		VH	2002
Santander	O	2	61		VH	2007
Litouwen	LA	3	59		VH	2005
Tresor	A	4	56		VH	1997
Casa Blanca	O	2	53	48	VH	1977
Pavia	LA	3	50		VH	2003

Yelloween	OT	3	50		WB	2005
Original Love	LA	3	41		Mak	2006
Dazzle	LA	3	40		Mak	1997
Nova Zembla	O	2	33		Mak	2001
White Heaven	L	2	38		WB	1999
Crystal Blanca	O	2	38		WB	1999
Star Gazer	O	2	37	437	Woodriff	1977
Merostar	O	2	37	18	IM	1991
Navona	A	3	34		VH	1994
Belladonna	OT	3	33		VH	2003
Corvara	O	2	32		Sande	2003
Acapulco	O	2	30	48	VH	1990
Triumphator	LO	3	30		RVZ	2003

The Dutch Flower Bulb Inspection Service has provided a list of the top 25 most widely grown lily hybrids (Table 1) according to the bulb hectares grown in the Netherlands in 2010 and 1994. This shows a comparison between the first *Lilium* symposium held in 1994 and 2010. This displays a transformation of the assortments. Merely five Oriental cultivars are included on both lists. The Number One lily grown for more than 25 years was 'Star Gazer' and in 1994 it was grown on 437 ha. This cultivar, having 7,300 ha grown since 1980 and 2010, is the top cultivar over time. Coming in 2nd place is 'Enchantment' (an Asiatic type) with 6,700 ha. A shift in the ranking is not expected.

Twenty hectares of the intersectional hybrids (LA, OT, LO) were cultivated in 1994 and increased to 1,290 hectare in 2010 (Table 2). Every cultivar on the list is protected by Plant Breeder's Rights with the

exception of 'Star Gazer'. Fresh lily cultivars can come quickly to market through tissue culture propagation. The largest contributors to the breeding arena are Vletter and Den Haan (recently merged with Marklily), Mak Breeding, Royal van Zanten, World Breeding and De Jong Lilies. Every one of the breeders is located in The Netherlands.

Breeding efforts are a major contributor to the development of the assortment in the future (Figure 2). With highly developed breeding methods, there has been a shift in the assortment over the last 15 years (Table 1). Asiatic hybrids have been substituted by LA-hybrids (in 2010 for 75%) and similarly the OT-hybrids may most likely replace the Orientals (in 2010 for 15%) and the LO-hybrids replacing the Longiflorums (in 2010 for 50%) (Table 2). The novel hybrids are triploid intersectional hybrids.

TABLE 2. Bulb production (hectares) of the main lily hybrid groups grown in The Netherlands in 1994 and 2010.

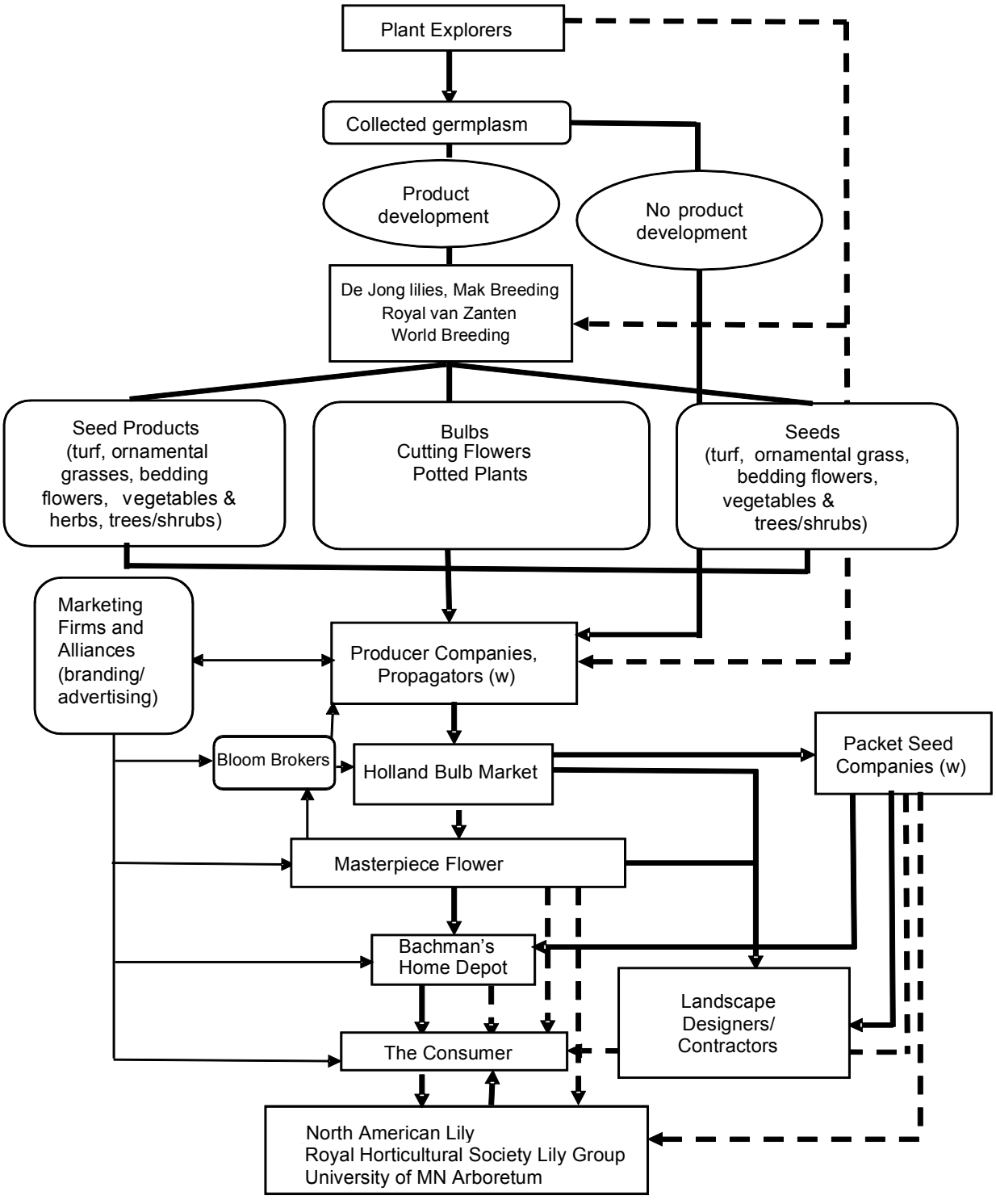
Hybrid group	1994	2010
Asiatic	1627 ha	436 ha
Oriental	1155	1799
Longiflorum	137	46
Longiflorum x Asiatic	21	886
Longiflorum x Oriental		53
Oriental x Trumpets		349
Total	3020	3624

In addition to the seven main hybrid groups (O, A, L, T, LA, LO and OT), other species have been used successfully in breeding efforts. Crossed species with the most potential to be used in new hybrid groups are: *L. candidum and L. monadelpum* (section *Lilium*), *L. pardalinum and L. canadense* (section *Pseudolirium), L. martagon* and *L. hansonii* (section *Martagon*), *L. nepalense* and *L. bakerianum* and *L. henryii*. Molecular cytogenetics (Lim, 2000; Barba-Gonzalez, 2005; Zhou, 2006; Khan, 2009) and molecular assisted breeding (Shanin et al., 2009) are methods that are being utilized to assist the introgression of characters from wild species to the assortment. Comprehensive genetic maps of two lily populations including 6 QTLs for *Fusarium*-resistance were published (Shain et al., 2010) recently.

Genetic transformation has been researched in lily for over 25 years (Cohen, 2011), and used in genetic studies. Up to the present time, transformation has not played a role in the development of new cultivars. Soon, knowledge of DNA-sequences linked to horticultural traits will be vital in connecting the genomes of lily. Enabling blending of desired complex traits from the wide genetic variation accessible to breeders.

FIGURE 2. The Horticulture Distribution Chain for lilies

(modified from Anderson,2015)



III. PRODUCTION INFORMATION

A. Current Production Practices.

The scheduling and timing of lily bulbs can vary. Frozen bulbs or bulbs produced in the other hemisphere may be forced any week of the year (Dole and Wilkins, 2004). De Hertogh (1996) compiled a flowering schedule for the 'Star Gazer' Oriental *Lilium* (Table 1). The schedule is for March to June forcing in the Northern Hemisphere. Forcing times vary greatly with each cultivar. The cultivar also determines the leaf number at a specific forcing date and flower development rate. There is a variance of shoot emergence from 2 to 3 weeks in the winter to 3 to 4 days for bulbs planted in April or after (B. Miller, personal communication). Orientals require 85 to 120 days of greenhouse forcing time with 50 to 55 days to flower from visible bud (De Hertogh, 1996; Lee and Roh, 2001). When the forcing season changes, temperatures influence leaf folding rate, flower bud development and flowering date (Lee and Roh, 2001; Roh, 1990a, Zhang et al 1990b). Acceptable for cut flower production is a high day temperature relative to the night temperature but not for potted plant production.

TABLE 1. Sample forcing schedule for 'Star Gazer,' a cut Oriental *Lilium* for Mother's Day.

Cultural Step	Production Time (Weeks)	Temperature °F (°C)
Receive bulbs from jobber and plant in crates or beds	7-8	Day: Within 5 (3) of night Night: 59 (15)
Visible bud, increase temperature	7-8	Day: Within 5 (3) of night Night: 63-65 (17-18)
Flower Total	14-16	
Bulbs must have 8 weeks of precooling, which is given prior to freezing at 28 – 30 °F (-2 to -1°C)		

B. Current Production Statistics.

The wholesale markets worldwide show different trends in lily sales (Figure 3). Flora-Holland, The Netherlands (ISPF 2014, from FloraHolland, 2013) had an increase in sales from 2011-212. Plantion, The Netherlands (ISPF 2014, from Plantion, 2012, 2013, 2014) was static from 2011-2012 but decreased in 2013. USA wholesale (APIH 2014, from Floriculture Crops 2013 Summary (June 2014), USDA, National Agricultural Statistics Service, 15-States-Program, Operations with $100,000+ Sales.) shows a downward trend from 2012 – 2013. The Taiwan China auctions (Floriculture Exporters Association (TFDA), Taiwan China) showed an increase in sales from 2012 – 2013. Note: Lily figures for Taiwan China are per bunches sold.

Eight countries are major players in lily production (Figure 4). The ranking is as follows from highest to lowest:

1) China (ISPF 2014, from China Flower Association, Statistics of China Floriculture Industry 2013, released by Ministry of Agriculture, PRC, July, 2014)
2) Kenya (ISPF 2014, from Horticultural Crops Development Authority (HCDA), Kenya)
3) Japan (ISPF 2014, from Statistics Department, Ministry of Agriculture, Forestry and Fisheries, Statistical Yearbook, Number 88, 2012/13)
4) Taiwan China (ISPF 2014, from Chinese Taipei Floriculture Development Association Yearly Report of Chinese Taipei Agriculture 2010, 2011, 2012)
5) Netherlands (ISPF 2014, from Centraal Bureau voor de Statistiek (CBS) and Bloembollenkeuringsdienst (BKS)
6) Mexico (ISPF 2014, from Servizio de informacion Agroalimentaria y Pesquera, Mexico)
7) USA (ISPF 2014, from 2012 Census of Agriculture, USDA, National Agricultural Statistics Service and USDA, Floricultural Crops 2013, Summary Data for Operations with $100,000+ Sales, 115 States Program)
8) Turkey (ISPF 2014, from Turkish Statistical Institute Database; Ministry of Food, Agriculture and Livestock)

FIGURE 3. Worldwide Wholesale Market Sales of Lilies (ISPF 2014)

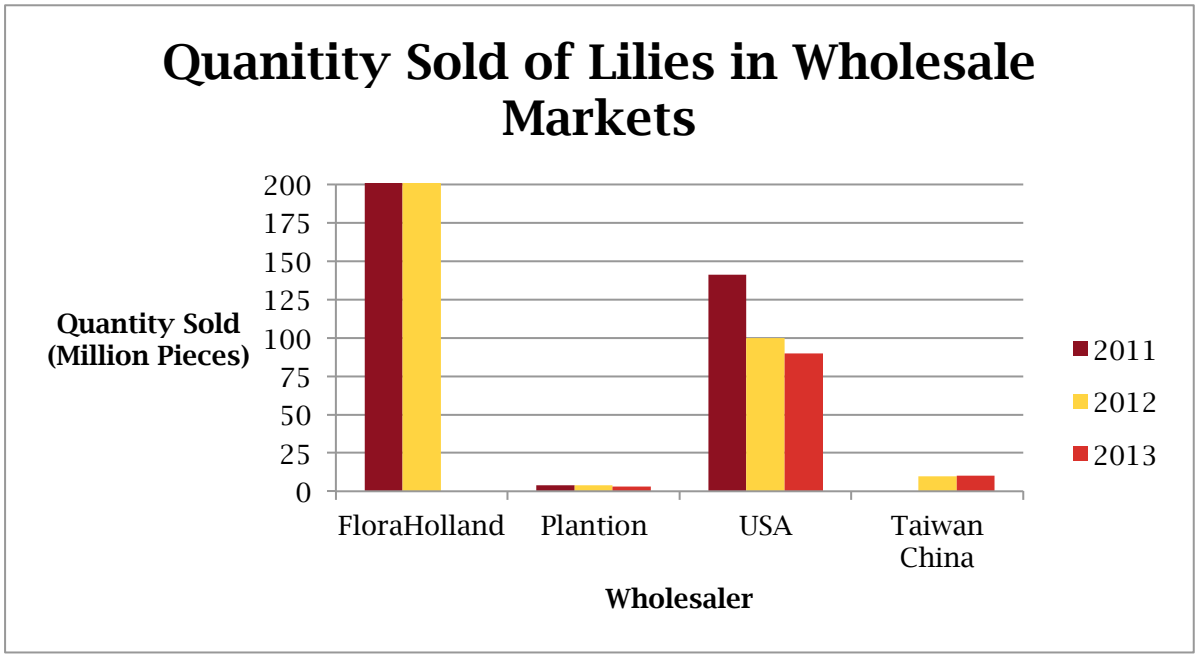
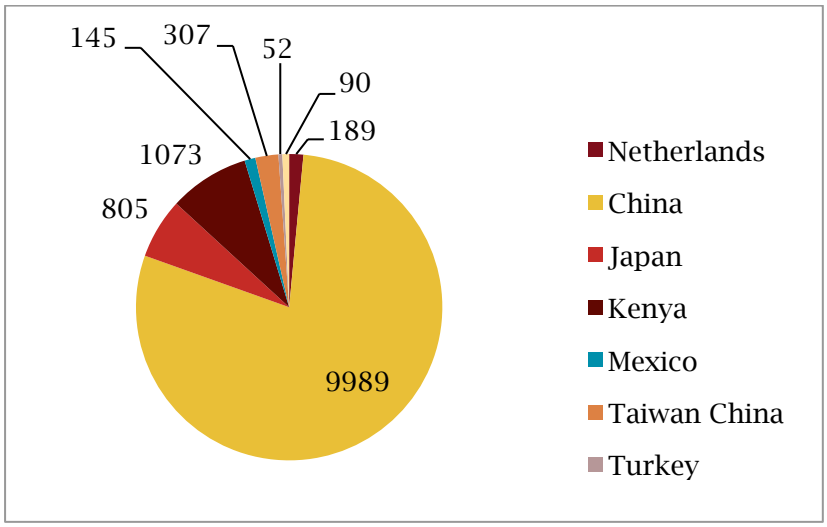


FIGURE 4. Lily Production, in Hectares, 2012 – 2013 (ISPF 2014)



Much breeding has been accomplished with Oriental lilies. The "LO Hybrids" are the newest addition of lilies. They are a cross between *L. longiflorum* and Oriental types. The graceful *L. longiflorum* leaves and enhancement of lower leaf characteristics was the intended goal of adding their genes to the gene pool The "OT Hybrids" are a cross between Oriental and Trumpet lilies (Dole and Wilkins, 2004). Oriental and OT lilies have fragrant flowers with some cultivars having a stronger scented flower than others (Gill et al., 2006). Double flowered Oriental cultivars have been developed (Dole and Wilkins, 2004). In the last 20 years, lily production has increased dramatically (De Hertogh, 2000). The traits breeders have

been focusing on are specific height and leaf characteristics, color range, improved root development during forcing, disease resistance in the field and greenhouse, increased flower number from small bulb sizes, tolerance to a wide variety of potential forcing and climate conditions (specifically for poor irradiance conditions which result in flower bud abortion), rapid and uniform forcing, decreased flower fragrance in the Oriental hybrids, improved flower substance and, particularly, postharvest performance (Lim et al., 2002: van der Muelen-Muisers et al., 1997). Another goal is to provide lily flowers with little or no pollen which has the positive effect of not staining clothing (Yamagishi, 2003).

IV. PROPOSED CROP TRANSFORMATION

A. Crop Production Change(s) for the Future.

The goal of this paper is to research the elimination of fragrance from Oriental lilies. The elimination of fragrance would have a wide range of potential markets. For the fresh cut flower industry this would benefit any individuals with allergies or sensitivity to strong smells who come in contact with the lilies. Since fresh cut flowers are put in floral arrangements this would have a target audience of hospitals, funeral arrangements, weddings and bouquets to the general public.

To enhance the demand for oriental hybrids the ability to control floral scent emission is desired (Fukai and Kawasaki, 2005). Mixtures of volatile compounds are responsible for the fragrance in flowers. They include aromatics, terpenoids, and nitrogen-containing compounds (Knudsen et al., 1993). These compounds are biosynthesized through pathways. The biosynthesis of aromatics is generally catalyzed by phenylalanine ammonia-lyase (PAL). Aminooxy acetic acid (AOA) and L-2-aminooxy-3-phenylpropionoic acid (AOPP) are known to be inhibitors of PAL (Oyama-Okubo et al., 2011). In an experiment by Naomi Oyama-Okubo, Masayoshi Nakayama and Kazou Ichimura tested the theory that biosynthesis inhibitors of aromatics might render the scent of the ‘Casa Blanca’ lily more pleasant by reducing the emissions of those compounds.

Lilium 'Casa Blanca' is an oriental hybrid cultivar which was introduced by Vletter & J.A. dem Haan Company in 1984 (Shimizu, 1987). With the lily containing a strong scent and large pure-white flowers, this made 'Casa Blanca' the ideal flower for use in the research. It was determined that the major fragrance compounds consisted of two aromatics (benzyl alcohol and *iso*-eugenol) and two terpenoids (linalool and *cis*-ocimene). Additionally p-cresol, p-creosol, vanillin and indole were distinguished as minor components. After two days of continuous application of 0.1 mM AOA or AOPP, to 'Casa Blanca' in vases, the concentrations of the main fragrance compounds (benzyl alcohol, *iso*-eugenol, linalool, *cis*-ocimene) were reduced by 10 – 20% in comparison to the control treatment. The total fragrance emissions of PAL inhibitor-treated flowers decrease to approximately 10% of the control. The fragrance of the AOA and AOPP treated lily was milder and more pleasant. There was no ornamental loss during the treatment (Oyama-Okubo et al., 2011).

Use of AOA or AOPP as a commercial fragrance production inhibitor may be viable. AOA is already being used as a preserving agent in cut flowers (Onozaki, 2002), is more accessible, and 400 times cheaper than AOPP. This makes AOPP the best option for scent reduction treatment in Oriental lilies. Post-harvest stems at late flower bud stage would be treated with 0.1 mM continuously for two days prior to shipment from the producers in the fresh cut flower sector.

Breeding efforts would also be a focus as a solution to fragrance elimination. It was mentioned earlier that some work has been done on this trait. So there is potential in this area of research along with interest. Crossing of Oriental lilies with Asiatic lilies may produce fragrance free lilies. Asiatic lilies have no fragrance (Rhee et al., 2005) so they would be a good place to start hybridizing. Interspecific crosses using Oriental x Asiatic hybrids have been obtained through embryo rescue and stigmatic and CSM pollination (Rhee et al., 2005). Thus, pursuing additional research in this area is promising. Breeding efforts will impact both fresh cut flower and the potted plant industry.

B. A New Production Schedule for Your Crop.

The crop schedule would be the same except for the addition of the AOA treatment two days prior to shipping. The recommended treatment time is at late bud stage so it possible that the application of AOA would be done by the flower markets. This treatment only affects fresh cut flower production.

C. The New Crop Ideotype.

Treatment of AOA would only be utilized in the fresh cut flower industry. New introductions could start out with the transformation of Oriental classics such as 'Casa Blanca' and 'Star Gazer'. The phenotype would be identical to the ones in the current marketplace but would offer decreased scent. The AOA process could be extended to include other Oriental hybrids such as OT and LO hybrids. This would provide consumers many options and expand the marketplace of fragrance free lilies. Additional research will be needed, with the goal of continuing to decrease the amount of volatile compound emissions in Oriental lilies; the ultimate goal being complete elimination of scent.

Breeding new cultivars of Oriental x Asiatic hybrids would affect the potted plant and fresh cut flower industries. The novel combinations would provide a mixture of new colors, sizes, and shapes of flowers that are also fragrance free. These new introductions provide a prime marketing opportunity. Nurseries could include them with current branding such as Proven Winners® or create new series like the Knock Out® Rose family of roses. Continuous breeding efforts would provide new plant options for consumers.

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