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INTRODUCTION

'Mid-Century' hybrid lilies are a new group of colorful, long blooming lilies. They require about three months from planting to blooming while <u>Lilium longiflorum</u> which has been used traditionally as the Easter lily needs four months.

'Mid-Century' hybrids have a wide range of colors (from yellow, orange, red, to deep crimson), large numbers of flowers (from 6 up to 20 buds per stem), and a blooming period of more than two weeks.

According to de Graaff and Hyams (1967), the <u>Lilium</u> x 'Mid-Century' hybrid lilies are basically an Asiatic group.

Among the ancestors of these hybrid lilies, <u>L. bulbiferum</u>

was the only European species that contributed to their genetic composition. The rest of the ancestoral species are of Asiatic origin.

The earliest ancestors of <u>L</u>. x 'Mid-Century' hybrids were <u>L</u>. <u>dauricum</u> and <u>L</u>. <u>concolor</u>. <u>L</u>. <u>dauricum</u> was native to northeast Asia, while <u>L</u>. <u>concolor</u> came from central China. The hybrid offspring of <u>L</u>. <u>dauricum</u> and <u>L</u>. <u>concolor</u> was <u>L</u>. x <u>maculatum</u>. This hybrid was introduced into Europe in the nineteenth century. Among the clones produced by <u>L</u>. x <u>maculatum</u> was one called 'Alice Wilson' which was a fashionable garden lily about 1880.

The next cross was \underline{L} . \underline{x} maculatum and \underline{L} . $\underline{bulbiferum}$. Their offspring was \underline{L} . \underline{x} hollandicum. \underline{L} . \underline{x} hollandicum was

then crossed with another Asiatic lily, \underline{L} . $\underline{tigrinum}$, in the United States. \underline{L} . \underline{x} \underline{umbtig} was obtained as the result of this crossing. \underline{L} . \underline{x} \underline{umbtig} then was used to cross with \underline{L} . \underline{x} 'Alice Wilson'. thus, \underline{L} . \underline{x} 'Mid-Century' hybrids were obtained (See Fig. 1).

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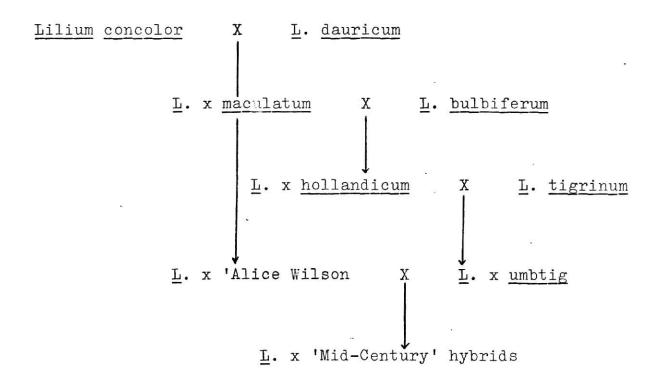


Fig. 1 Origin of 'Mid-Century' Hybrids. (de Graaff, 1967)

LITERATURE REVIEW

Both self- and cross-incompatibility are frequently found in <u>Lilium</u> species. Often, after self- or cross-pollination, no seed is obtained. The genetic basis of self-incompatibility in <u>Lilium</u> was suggested as resembling that of a gametophytic type by Ascher and Peloquin (1966 c).

Ascher (1966) used a gene action model to explain gametophytic self-incompatibility in <u>Lilium</u>. He assumed the S allele produces monomers of a dimer repressor controlling a high rate of growth operon in the pollen tube. When similarity between monomers in the style and pollen tube occurs, a functional dimer repressor is produced which inhibits this operon yielding pollen tube growth typical of incompatibility. Pollen tubes would function when the high velocity operon is inactive.

Endosperm-embryo incompatibility has been found in <u>Lilium</u> species. Emsweller and Uhring (1958) found that pollinations involving <u>Lilium speciosum album x L. auratum</u> produced a relatively large number of plump seeds with embryos. All seeds failed to form seedlings when planted in soil or in vermiculite. Unplanted seeds were examined and it was found that the embryos were partially digested in the endosperm. They repeated the pollinations and embryos from green seed pod capsules at various stages of maturity were excised and grown in nutrient agar. Over 500 hybrid seedlings were obtained from excised embryos. They stated "the results indicate some

type of endosperm-embryo incompatibility that can be overcome by excising embryos before maturity and growing them on nutrient agar".

Ascher and Drewlow (in press) found unilateral interspecific incompatibility appeared to be common in crosses of Lilium longiflorum. They observed nearly all of the lilies exhibited large differences in pollen tube growth in reciprocal pollinations with L. longiflorum. Only 'Enchantment', 'Discovery' and L. henryi showed exceptions. Mean pollen tube length measured after 48 hours incubation of detached pollinated styles of L. longiflorum x L. regale was 6.6 mm, while in the reciprocal cross it was found to be 80.9 mm. However, they observed only 41.1 mm in the cross of L. longiflorum x L. 'Enchantment' and 11.0 mm of pollen tube growth in the reciprocal pollination. Their analysis of pollen tube growth indicated that the Easter lily should be used as the pollen donor for hybridization of trumpet type lilies to increase seed yield.

Hopper and Peloquin (1968) used X-rays to cause inactivation of the stylar component of the self-incompatibility reaction in \underline{L} . longiflorum. They found X-irradiation prior to pollination of the excised style had a significant effect on self-pollen tube growth. They reported growth rate of incompatible pollen tubes in excised styles exposed to dosages of 24 to 70 Kr. was as high as that of compatible tubes growing in untreated styles. They also

found a corresponding increase in pollen growth rate following 6 to 24 Kr exposure of the style.

Lewis (1942) reported that \underline{L} . grandiflorum compatible pollen tubes showed an increased rate of growth with increased temperatures and had an optimum growth rate between 15 and 20° C. Pollen tubes showed less growth at room temperatures and even less at progressively higher temperatures.

Conversely, Ascher and Peloquin (1966 a) found a different situation in L. longiflorum. They grew compatible and incompatible pollen tubes in detached styles of four cultivars of L.longiflorum at five temperatures, ranging from 11 to 39° C. They observed in a 48-hour incubation period that compatible pollen tubes increased in length with increasing temperature up to 30° C, but did not traverse the length of the style. At both 30° and 39° C they traversed the length of the style and reached the top of the ovary. They also found incompatible pollen tubes showing typical inhibition only at room temperature (24° C). Incompatible pollen tubes grew completely through the style and were indistinguishable from compatible tubes at 39° C. Incompatible pollen tubes at 19° C were somewhat shorter than compatible. No distinction could be made between compatible and incompatible pollen tubes at 11° C. They suggested that the breakdown of the incompatibility reaction at high temperatures might occur primarily in the style. The similar growth rate of both types of pollen tubes at low temperatures seemed more likely to be

the influence of low temperatures on pollen tube metabolism rather than on the style.

Emsweller and Uhring (1965) applied a growth regulator to self-pollinated Creole lilies grown in five temperatures of 11.1°, 16.7°, 22.2°, 27.8°, and 33.3° C. The growth regulator was a 1% solution of naphthalene acetamide in lanolin and was applied to the wound made by removing a petal. They obtained self-seed set following application of the growth regulator at the time of pollination and within a temperature range of 12.8° to 18.3° C. Their results are similar to the findings of Lewis but differ from those of Ascher and Peloquin. The difference probably is due to the interaction of temperature and growth regulator.

Hopper and Peloquin (1967) reported that pretreatment in distilled water at 50° C for six minutes immediately prior to self-pollination significantly decreased inhibition of incompatible pollen tube growth in <u>L. longiflorum</u>. They found incompatible pollen tubes attained an average length of 74 mm when incubated for 48 hours at 24° C following stylar pretreatment at 50° C. In contrast, an average tube length of 50 mm occurred when styles were pretreated at 25° C.

In their further experiment, Hopper and Peloquin (1968) found that at increasing heat treatment time intervals of 1, 2, 3, 4, 5, and 6 minutes before pollination, the response of inactivation of self-incompatibility increased. The similar response following both X-irradiation and heat treatment; i. e.,

the effect increased as dosage increased, led them to assume that there was an enzyme-inactivation effect involved.

Emsweller, Uhring, and Stuart (1960) used naphthalene acetamide and potassium gibberellate at the time of self-pollination of L. longiflorum to induce self-compatibility. They reported this treatment caused delayed senescence of the embryo sacs for the 26 days of the test period. At which time ovules in the untreated capsules were completely shrivelled. Pollen tubes were observed in the micropyle of several embryo sacs 17 days after pollination. Treated capsules increased in length and diameter during the 26 day period. They reported that naphthalene acetamide used alone or in combination with potassium gibberellate greatly increased seed set.

Ascher and Peloquin (1966 b) observed pseudo-compatibility in self-incompatible <u>L</u>. <u>longiflorum</u> 'Ace' after either late pollination (six or more days after anthesis) or application of naphthalene acetic acid to the receptacle at the time of pollination. In a period from six to ten days after anthesis, self-pollination resulted in the same percentage of ripened fruits as cross-pollination, indicating that self-pollination tubes were able to traverse the style regularly. The number of seeds per capsule was about half that obtained by cross pollination. They found there were about ten times as many seeds per fruit with late selfing as with naphthalene acetic acid treatment. They suggested that the relatively large

quantity of self-seeds after late pollination was due to stylar conditioned pseudo-compatibility. This pseudo-compatibility resulted from a stylar deficiency which was induced by aging and permitted self-incompatible pollen tubes to grow. In contrast, growth substance treatment allowed measurement of the capacity of individual pollen tubes to escape inhibition by retaining fruits on the plant regardless of the number of seeds they hold. Thus, they suggested that self-seed set after growth substance treatment was an expression of pollen-conditioned pseudo-compatibility.

Assuming slow pollen tube growth as a factor influencing self-incompatibility and some of the cross-incompatibility in Lilium, Sagawa (1959) reported that self-incompatibility of L. longiflorum could be overcome by pollination of 2 cm excised styles. Limited seed set was obtained using this procedure. Watts (1967) used intrastylar pollination to obtain seed set of some selfs and wide crosses of lilies. In his study, intrastylar pollination was accomplished by cutting the style at a slant, about halfway between the stigma and ovary, then placing pollen in the base of the stylar canal thus created.

Watts reported that intrastylar pollination did induce or improve more seed set than stigmatic pollination. By using this technique in crossing 'Cinnabar' with 'Golden Chalice' 51.1 seeds per fruit were obtained, while in case of stigmatic pollination no seed was obtained. He also reported in cases

where no seed was set following either stigmatic or intrastylar pollination, parthenocarpic fruit development was . increased by intrastylar pollination.

The objectives of this thesis were to determine the effect of different intrastylar pollination methods on obtaining self- and cross-seed set of the 'Mid-Century' hybrid lilies. A complete 4 x 4 diallel crossing pattern was used to evaluate compatibility and seed set. The relationship between seed number, pod width, pod length, and maturity was also under investigation.

Results of this study were written in manuscript form to be submitted for publication in the Journal of the American Society for Horticultural Science.

LITERATURE CITATION

- 1. Ascher, P. D. 1966. A gene action model to explain gametophytic self-incompatibility. Euphytica 15: 179-183.
- and S. J. Peloquin. 1966 a. Influence of temperature on incompatible and compatible pollen tube growth in L. longiflorum. Canad. J. Genet. Cytol. 8: 661-664.
- 3. and . 1966 b. Pseudocompatibility in self-incompatible <u>L</u>. longiflorum. <u>Proc</u>. XVII. Int. Hort. Cong. 1: 206.
- 4. and . 1966 c. Effect of floral aging on the growth of compatible and incompatible pollen tubes in <u>L. longiflorum</u>. Amer. J. Bot. 53 (1): 99-102.
- 5. and L. W. Drewlow. Unilateral interspecific incompatibility in Lilium. In press.
- 6. de Graaff, J. and E. Hyams. 1967. pp. 71-78. <u>In</u>: Lilies. Funk and Wagnalls.
- 7. Emsweller, S. L. and J. Uhring. 1958. Endosperm-embryo incompatibility in <u>Lilium</u> species hybrids. <u>Proc. 15th</u>. <u>Inter. Hort. Cong.</u>, Nice, France. <u>2</u>: 360-367.
- 8. and . 1965. Interaction of temperature and growth regulator in overcoming self-incompatibility in Lilium longiflorum. Hereditas 52: 295-306.
- 9. and N. W. Stuart. 1960. The roles of naphthalene acetamide and potassium gibberellate in overcoming self-incompatibility in Lilium longiflorum. Proc. Amer. Soc. Hort. Sci. 75: 720-725.
- 10. Hopper, J. E., P. D. Ascher, and S. J. Peloquin. 1967. Inactivation of self-incompatibility following temperature pretreatments of styles in <u>Lilium longiflorum</u>. <u>Euphytica</u> 16: 215-220.
- and S. J. Peloquin. 1968. X-ray inactivation of the stylar component of the self-incompatibility reaction in Lilium longiflorum. Canad. J. Genet. Cytol. 10: 941-944.
- 12. Lewis, D. 1942. Physiology of incompatibility in plants.

- I---The effect of temperature. Proc. Roy. Soc. (London) B. $\underline{131}$: 13-26.
- 13. Sagawa, Y. 1959. Self-incompatibility among Easter lilies. Proc. IX Int. Bot. Cong. 339. Abstract.
- 14. Watts, V. M. 1967. Influence of intrastylar pollination on seed set in lilies. <u>Proc. Amer. Soc. Hort. Sci. 91</u>: 660-663.

EFFECT OF INTRASTYLAR POLLINATION METHODS ON SEED SET OF 'MID-CENTURY' HYBRID LILIES

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Abstract: A complete 4 x 4 diallel showed significant self-incompatibility and reciprocal differences in 'Mid-Century' hybrid lily crosses. For all crosses, normal stigmatic pollination produced more seed set than either intrastylar pollination method. For selfed flowers, both intrastylar pollination methods produced non-significant increases in seed set. Significant reciprocal differences were found between crosses of 'Harmony', 'Cinnabar', and 'Enchantment'. No reciprocal differences existed between 'Joan Evans' crosses. Seed set was highly correlated with pod width, length, and days to maturity.

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INTRODUCTION

'Mid-Century' hybrid lilies range in color from yellow, orange, red to deep crimson. They produce from six to twenty flowers per stem during a blooming period of more than two weeks. 'Mid-Century' hybrids force one month earlier than Easter lilies (2). These hybrid lilies can be used as pot plants, cut flowers, or as hardy garden flowers, but their full genetic potential has yet to be realized.

Self-incompatibility has been a problem for genetic studies in <u>Lilium</u>. Emsweller and Stuart (3) reported that slow pollen tube growth was a factor influencing self-incompatibility in <u>L. longiflorum</u>. Sagawa (5) showed that <u>L. longiflorum</u> self-incompatibility could be overcome by pollinating styles excised at 2 cm. Watts (6) reported that intrastylar pollination shortened the distance for pollen tube growth and that seed set of some self and wide crosses of <u>Lilium</u> was induced.

Literature regarding the 'Mid-Century' hybrid lilies and their genetic incompatibility systems is limited. This research examined three pollination methods and compared the self-incompatibility and reciprocal differences of 'Mid-Century' hybrid lilies using a complete 4 x 4 diallel.

· MATERIALS AND METHODS

The 'Mid-Century' hybrid lilies 'Harmony', 'Cinnabar',
'Enchantment', and 'Joan Evans' were obtained from Oregon Bulb
Farm, Gresham, Oregon. On October 3, 1970, the 7 to 8-inch
circumference bulbs were placed in 6-inch pots containing a
soil mix of equal parts of sandy loam, peat moss, and haydite.
The bulbs were precooled in the pots at 40° F until November
16 when they were moved to the greenhouse for forcing at 60° F
night temperatures.

A complete diallel which included all reciprocal cross and selfs was made using the four 'Mid-Century' hybrids. Within each of the 16 possible selfs or crosses, three methods of pollination were used. Each pollination method was replicated five times giving a total of 240 pollinations.

Normal pollination consisted of placement of pollen on the stigmatic surface. Cut intrastylar pollination was done by cutting the style approximately one cm above the ovary with a clean razor blade leaving only enough stylar canal in which to place pollen. Intrastylar implant pollination was accomplished by cutting a one cm longitudinal slit halfway up the style and packing pollen into the opening with an alcohol sterilized needle. With all pollinations, the anthers and petals were removed and the ovary was covered with aluminum foil.

Pollinations were made from January 29, 1971 ('Harmony') to February 9, 1971 ('Joan Evans'). Pods were harvested before seed dehisced in May. Length and width of seed pods were

measured to the nearest centimeter. Days from pollination to harvest were recorded. A constant air stream was used to separate viable (heavy) seed from nonviable (light) seed. Seed count were made and germination tests confirmed the viability of heavy seeds.

Analysis of variance, least significant differences, and simple correlations were calculated with an IBM 360/50 computer for seed number, seed pod characteristics, and maturity data.

RESULTS AND DISCUSSION

Average performance of the four cultivars varied (<u>Table 1</u>).

'Cinnabar' produced the greatest seed number (64.8), but was the worst pollen parent (25.8 seeds/pod). 'Enchantment' was a good pollen parent (50.1 seeds/pod) and the poorest seed parent (19.5). 'Harmony' produced 32.7 and 41.1 as seed and pollen, respectively. 'Joan Evans' had identically 43.2 seeds as \(\frac{2}{3} \) and \(\frac{5}{3} \). None of the crosses involving 'Joan Evans' produced significant reciprocal differences.

As shown in <u>Table 2</u>, significant reciprocal differences occurred between crosses of 'Harmony', 'Cinnabar', and 'Enchantment'. It was assumed that style lengths were similar for all stigmatic pollinations ranging from 3 to 4 cm. All reciprocal crosses were made within short time intervals so that environmental differences were minimal. Reciprocal differences may have been caused by embryo-endosperm incompatibility as described by Emsweller and Uhring (4) or

Table 1. Average performance of four 'Mid-Century' hybrid lilies expressed as seed number/pod for all pollination methods.

3 0	Seed parent	Pollen par	rent
	cross	cross	self
'Cinnabar'	64.8	25.8	7.1
'Joan Evans'	43.2	43.2	9.0
'Harmony'	. 32.7	41.1	3.3
'Enchantment'	19.5	50.1	0.1

Table 2. Effect of cultivar x pollination method on mean number of seed set per capsule of 'Mid-Century' hybrid lilies in a complete 4 x 4 diallel.

Pare	nts*	Pollination method			Mean
우	\$	Stigmatic	Stylar cut	Stylar implant	
C X C X JE X JE X H X E X JE X H X E X C X E X H X E X	E JE C E JE H JE C H JC C H	140.8 138.8 137.0 91.6 106.2 106.0 108.4 87.2 48.4 35.4 35.2 1.6 0.0 20.4 0.0	42.0 22.2 17.4 29.6 11.6 7.8 0.0 10.4 19.2 12.4 0.0 13.4 12.2 0.6 10.0 0.4	41.0 21.0 23.2 23.2 18.4 18.6 8.4 10.4 26.8 19.4 2.8 12.0 9.0 0.0	74.6 60.7 59.1 45.4 44.1 36.5 31.5 22.7 77.0 3.1
LSD	(0.01)	32.4	32.4	32.4	18.7

* H = 'Harmony'
C = 'Cinnabar'
E = 'Enchantment'
JE = 'Joan Evans'

by other factors.

All four 'Mid-Century' hybrid lilies expressed a high degree of self-incompatibility. Except for 'Harmony' x 'Cinnabar', 'Enchantment' x 'Harmony', and 'Enchantment' x 'Cinnabar', all crosses yielded significantly more seed than self pollinations.

'Mid-Century' hybrid lilies since all crosses produced fairly high seed counts using normal stigmatic pollination methods. Both intrastylar pollination methods significantly reduced seed set for most crosses. It is possible that unfavorable physiological changes occurred within the wounded styles. Physiological stresses such as loss of nutrients, water, or other factors may have occurred from the excised style. It is also possible that some essential metabolite may normally be present in the stigmatic surface which preconditions the growth of pollen tubes down the stylar canal.

The cut and implant intrastylar pollinations did not result in partial correction of cross-incompatibilities; i. e., ('Enchantment' x 'Cinnabar'). This is in disagreement with Watts (6) who showed that intrastylar pollination improved seed set in cases of cross-incompatibility.

As shown in <u>Table 3</u>, seed number and pod development were significantly influenced by method of pollination. Normal stigmatic pollination yielded significantly more seed, larger pod size, and were slower to develop than either intrastylar

pollination method.

Simple correlation tests showed highly significant relationships between seed number, pod width, pod length, and maturity (<u>Table 4</u>). Larger seed pods contained more seeds and required more time to mature.

From these results, it can be assumed that self-incompatibility will be a problem in breeding work with 'Mid-Century' hybrid lilies. Intrastylar pollination can be used as a technique to overcome self-incompatibility, but the small increase in seed set requires considerable time.

Table 3. Effects of pollination methods on seed number and pod size (mean of all five replications).

Pollination method	Seed number	Pod width (cm)	Pod length (cm)	Maturity (days)
Crosses				
Stigmatic Stylar cut Stylar implant Selfs	88.0 14.4 17.8	1.8 1.1 1.2	4.1 3.1 3.2	79.4 70.6 71.5
Stigmatic Stylar cut Stylar implant	0.4 9.0 5.3	0.6 1.0 0.9	2.5 3.0 2.7	69.0 69.7 69.1
LSD (0.01)				-
Crosses Selfs	9.3 16.1	0.2 0.3	0.3 0.5	3.1 5.7

Table 4. Phenotypic correlations between seed pod characteristics and maturity time in days of 'Mid-Century' hybrids.

_	Width	Length	Seed number
Length	.901**	**	-
Seed number	.745**	.711**	- **
Maturity	.585	.489	•533

^{** = 1%} level of significance. total observations = 240

LITERATURE CITED

- 1. Ascher, P. D. and S. J. Peloquin. 1968. Pollen tube growth and incompatibility following intra- and interspecific pollinations in <u>Lilium longiflorum</u>. <u>Amer. J. Bot. 55</u>: 1230-1234.
- 2. de Graaff, J. 1970. Recommended culture for forcing 'Mid-Century' lilies. Oregon Bulb Farm. Gresham, Oregon.
- 3. Emsweller, S. L. and N. W. Stuart. 1948. Overcoming sterilities in <u>Lilium</u> by the use of growth regulating substances. North American Lily Soc. Yearbook 1: 27-30.
- 4. and J. Uhring. 1962. Endosperm-embryo incompatibility in <u>Lilium</u> species hybrids. <u>In</u>: Advances in Horticultural Science and Their Applications. Pergamon Press.
- 5. Sagawa, Y. 1959. Self-incompatibility among Easter lilies. Abstract. <u>Proc. IX Int. Bot. Congress</u> 339.
- 6. Watts, V. M. 1967. Influence of intrastylar pollination on seed set in lilies. <u>Proc. Amer. Soc. Hort. Sci. 91</u>: 660-663.

APPENDIX

EXPERIMENTAL PROCEDURES:

Greenhouse

The experiment was conducted in section 141 on north-south raised bench on the east side of the upper Horticulture and Forestry Greenhouses, Kansas State University campus.

Plant Materials

Twenty five bulbs each of 'Harmony' and 'Enchantment', thirty of 'Cinnabar' and fifteen of 'Joan Evans' were purchased from Oregon Bulb Farm, Gresham, Oregon. All bulbs of the four cultivars were of seven to eight-inch circumference.

Potting Medium

The soil mix consisted of one part sandy loam, one part peat moss, one part haydite. Before potting, sphagnum moss was placed in the bottom of a six-inch pot. Soil mix was added to fill one half the pot. Bulb was planted and covered with approximately one inch of soil. The plants were watered thoroughly after potting.

<u>Handling</u>

The bulbs were obtained and potted on October 3, 1970. Each bulb of each cultivar was coded by receiving cultivar name initial and a randomly chosen arabic numeral. They were stacked pot to pot in a 40° F cooler and precooled from October 3 to November 16 (six weeks). On November 16, they were moved to the 60° night temperature greenhouse. They were

randomly placed, and spaced at approximately twelve to fifteen inches between pots, on the bench.

Culture

The plants were watered daily or as the need occurred with a 20-20-20 soluble fertilizer at approximately 100 ppm nitrate-nitrogen. Natural lighting as well as partial shading on greenhouse roof was provided.

Pests and Insects Control

To avoid interaction of insecticides and chemicals with seed set, plants were not sprayed during seed development.

Some red spider mites were noted late in the study, but were not considered to have significantly influenced lily growth.

Pollination Procedure

The fastest developed cultivar was 'Harmony' which bloomed first on January 29, 1971. The second one was 'Cinnabar' which bloomed on January 30 and the third one was 'Enchantment', on February 6. The latest one was 'Joan Evans' which showed first blooming on February 9, 1971. To avoid 'Harmony' and 'Cinnabar' blooming out before making crosses with 'Enchantment' and 'Joan Evans', some plants of these two cultivars were moved to a 55° F night temperature glasshouse for two weeks to slow down their development while 'Joan Evans' and 'Enchantment' were still kept at 60° F night temperatures. 'Joan Evans' still developed very slowly, so several slower developed plants of 'Harmony' and 'Cinnabar' were moved further to the 40° F cooler. Furthermore, pollen

from randomly chosen flowers of the later two cultivars were stored in alcohol sterilized vials at 40° F to assure adequate amount of pollen for crossing.

Because there were no growth differences due to plants, flower buds of each cultivar chosen for pollination either as pollen or seed parents were randomly selected. Flower buds were first emasculated by removing all anthers and petals one day before anthesis to prevent natural cross pollinations. Due to warm temperature in the middle of a day (from 10AM to 3 PM approximately), stigmatic exudate was easily dried out. To assure successful pollination, pollination was done after emasculation in the early morning (before 10 AM) or late afternoon (after 3 PM). After each pollination, the bud was wrapped with a piece of aluminum foil to avoid contamination and tied with a tag of both parents names and date of pollination. (As each plant received a random number, whenever a name tag was written, the code number of both parents was also recorded so that whether there was any difference in seed set due to different plants could be examined.) The aluminum foil was removed one week later when the ovary began to swell.

Normal and intrastylar pollination techniques are discussed in the manuscript. A syringe pollination technique was also exercised. Pollen was collected from each cultivar and placed in a small vial. The pollen grains were dispersed in a few drops of tap water. An alcohol sterilized

hypodermic syringe was used to inject the pollen suspension through the stigma into the style. Rapid injection resulted in damage to stylar tissues. Then the bud was wrapped with aluminum foil and tied with a name tag with pollination date and the cross recorded as discussed above.

Data Recording and Harvesting

When the flower buds began to swell and were clearly visible, bud count per bulb and number of stems per bulb were recorded. Average bud count per stem was taken by dividing total bud count by total number of stems. Plant characteristics were also recorded (Table 5). Both pollination and harvest date were recorded and maturity days from pollination to harvest were counted.

Seed pods were harvested before dehiscence, i. e., before pods showed dark brown color and seeds dispersed. Pods were cut off with a clean razor blade. Length and width of seed pods were measured with a ruler to the nearest centimeter. Longitudinal length from the bottom to the top of seed pod was measured. Diameter of the pod was taken as width.

Mature seed pods were harvested. Heavy and light seeds were separated by a constant air stream. Heavy seeds were counted and recorded as seed number variable. Germination tests were made in a 65° F, 65% RH and artificial lighting growth room by putting both heavy and light seeds into separated lines on a piece of wet filter paper in a petri dish. Heavy seeds germinated while light ones didn't. Thus

the tests confirmed the viability of heavy seeds. Statistics

Statistical analysis was done to determine the effect of pollination method on seed pod width (in cm), seed pod length (in cm), and seeds maturity time (in days). Means are presented in Tables 6-8. Seed pod width, length and maturity were highly correlated with mean seed number (<u>Table 2</u> and <u>4</u>). Therefore, Tables 6-8 which showed similar trends as Table 2 were not presented in the manuscript.

Analysis of variance including degrees of freedom, mean squares and F values of seed pod width, seed pod length, seed number and maturity time in days are presented in Table 9-

As shown in Table 13, syringe pollination technique almost secured the same amount of seed set as intrastylar pollination method. This table is based on data of the pollinations made from February 12-16, 1971.

Table 5. General information of 'Mid-Century' hybrid lilies.

	Cultivar				
	'Harmony'	'Cinnabar'	'Enchantment'	'Joan Evans'	
Color	Rich orange with dark brown spots.	Deep crimson with dark brown spots.	Nasturtium red with dark brown spots.	Soft orange with dark brown spots.	
Average bud count per stem	15	7	18	8	
Number of stems per bulb	1	1	1	2	
Total bud count per bulb	15	7	18	16	
Bulblets	Many	Few	Many	Many	
Other characte- ristics of plants	Glabrous leaves but unattractive arrangement.	Pubescent leaves and flower buds. Leaf arrange- ment attrac- tive.	Tall stems with glabrous large leaves. Good leaf arrangement. Tends to produce two flowers per flower spike at basal part of florescence.	Short and weak stems with glabrous leaves and flower buds. Flowers produced in umbel.	

Effect of pollination method on seed pod width of 'Mid-Century' hybrid lilies. (in cm) Table 6.

Parer	nts*	Pollination method			Mean
7	\$	Stigmatic	Stylar cut	Stylar implant	2
C X X X X X X X X X X X X X X X X X X X	H JE CEEHCEJC JE H EH	2.3 2.1 2.1 2.0 1.9 1.2 2.1 1.3 0.4 1.7 1.1 1.2 0.4 0.2	1.8 1.6 1.6 1.4 1.2 1.0 1.4 0.8 1.0 0.8 1.3 0.7 0.4 0.6 0.6	2.0 1.6 1.5 1.3 1.4 1.1 1.4 1.0 1.3 1.1 1.2 0.8 0.7 0.6 0.5 0.3	2.0 1.8 1.7 1.6 1.5 1.3 1.3 1.2 1.1 1.0 0.8 0.8 0.5
ĿSD	(0.01)	0.7	0.7	0.7	0.4

* H

'Harmony'
'Enchantment'
'Cinnabar' \mathbf{E} C JE = 'Joan Evans'

Table 7. Effect of pollination method on seed pod length of 'Mid-Century' hybrid lilies. (in cm)

Parent	* S		Pollination method		
우	\$	Stigmatic	Stylar cut	Stylar implant	_ Mean
C X C X JE X JE X H X JE X E X H X H X H X H X H X H X H X H X	H E JE C E H JE C JE H E H	4.5 5.0 4.3 4.2 3.2 4.0 5.2 6.3 7.3 0 2.3 2.0	4.3 4.0 3.8 3.2 3.8 2.9 2.8 2.9 2.9 2.3 2.3 2.6	3.7 3.6 3.9 3.2 3.1 2.8 3.1 2.9 3.2 2.9 3.7 2.4	4.2 4.1 3.6 5.5 3.4 3.2 3.1 3.0 2.4 2.1
LSD	(0.01)	1.0	1.0	1.0	0.6

H =

'Harmony'
'Enchantment'

'Cinnabar'

JE = 'Joan Evans'

Effect of pollination method on seeds maturity time of 'Mid-Century' hybrid lilies. (in days) Table 8.

Pare	nts*		Pollination method		
9	8	Stigmatic	Stylar cut	Stylar implant	Mean
JE JE C C C H H H H C E E E	X C X JI X E X H X C X JI X C X JI X H X C X JI X C X JI X C X JI X C X C X JI X C X C X C X C X C X C X C X C X C X	84.8 84.0 83.2 86.0 85.2 76.8 78.2 85.6 70.4 66.6	81.0 85.6 79.6 77.0 79.4 73.4 72.6 72.4 72.0 67.8 66.8 69.0 49.6 62.4 59.4	81.0 77.4 82.4 77.0 71.0 73.0 74.6 77.8 76.2 72.0 76.2 70.0 61.4 54.6 56.4	82.6 82.3 82.4 79.3 77.5 75.7 75.7 75.1 71.5 61.4 59.9
LSD	(0.0	11.5	11.5	11.5	6.6

^{*} H: =

^{&#}x27;Harmony'
'Enchantment'

^{&#}x27;Cinnabar'

^{&#}x27;Joan Evans' JE =

Table 9. Analysis of variance of seed pod width	Table 9.	Analysis	of varianc	e of seed	pod width
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	Source of variance	Degrees of freedom	Mean square	F value
3.	Genotype Methods Genotype x Methods Error Total	15 2 30 192 239	3.20 4.01 0.58 0.18	18.11** 22.71* 3.29*

Significance at 1% level. F 0.01 = 1.70 Highly significance at 1% level. F 0.01 = 2.04 Very highly significance at 1% level. F 0.01 = 4.61***

Table 10. Analysis of variance of seed pod length.

Source of variance	Degrees of freedom	Mean square	F value
Genotype Methods Genotype x Methods Error Total	15 2 30 192 239	5.05 8.42 0.99 0.37	13.79*** 22.99* 2.71

Significance at 1% level. F 0.01 = 1.70 Highly significance at 1% level. F 0.01 = 2.04**

Very highly significance at 1% level. F 0.01 = 4.61

Table 11. Analysis of variance of seed number	Table	11.	Analysis	of	variance	of	seed	number
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Source of variance	Degrees of freedom	Mean square	F value
Genotype Methods Genotype x Methods Error Total	15 2 30 192 239	8097.84 75728.44 3861.10 394.42	20.53*** 184.39* 9.79

Analysis of variance of maturity time in days. Table 12.

Source of variance	Degrees of freedom	Mean square	F value
Genotype Methods Genotype x Methods Error Total	15 2 30 192 239	1184.78 1036.52 102.96 49.40	23.98** 20.98* 2.08

Significance at 1% level. F 0.01 = 1.70

Significance at 1% level. F 0.01 = 1.70 Highly significance at 1% level. F 0.01 = 2.04

Very highly significance at 1% level. F 0.01 = 4.61

Highly significance at 1% level. F 0.01 = 4.61

Very highly significance at 1% level. F 0.01 = 2.04

Table 13. Effect of four pollination methods on seed set of 'Joan Evans' crosses.

	Number of pollinations	Average seed set
'Joan Evans' x 'Enchantment'		
Normal stigmatic Stylar cut Stylar implant Syringe	3 4 4 4	107.0 54.3 36.7 45.7
'Joan Evans' x 'Harmony'	8	
Normal stigmatic Stylar cut Stylar implant Syringe	6 4 5 4	93.7 44.7 28.5 62.0
'Joan Evans' x 'Cinnabar'		
Normal stigmatic Stylar cut Stylar implant Syringe	5 6 6 6	75.8 46.0 28.8 12.7

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POLLINATION STUDIES OF 'MID-CENTURY' HYBRID LILIES

by

MING-YUAN HSU

B. S. National Taiwan University, Taipei, China, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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MASTER OF SCIENCE

Department of Horticulture & Forestry

KANSAS STATE UNIVERSITY Manhattan, Kansas

A complete 4 x 4 diallel showed significant selfincompatibility and reciprocal differences in 'Mid-Century'
hybrid lily crosses. For all crosses, normal stigmatic
pollination produced more seed set than either intrastylar
pollination method (stylar cut and stylar implant). For
selfed flowers, both intrastylar pollination methods produced
non-significant increases in seed set over normal pollination.
Injection of a pollen-water suspension into the style with a
hyperdermic syringe gave similar seed set as intrastylar
pollination methods.

Significant reciprocal differences were found between crosses of 'Harmony', 'Cinnabar', and 'Enchantment'. No reciprocal differences occurred between 'Joan Evans' crosses. Seed set was highly correlated with pod width, length, and days to maturity. Germination tests confirmed that the heavy seeds counted were viable.