

SOME EFFECTS OF PHOTOPERIOD AND ALTERNATING TEMPERATURE
ON ROOT AND SHOOT DEVELOPMENT OF LILIUM TIGRINUM BULBILS

by

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B. S., Fort Hays Kansas State College, 1968

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture and Forestry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1973

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TABLE OF CONTENTS

	PAGE
INTRODUCTION.....	1
LITERATURE REVIEW.....	2
Aerial bulbils	
Occurrence.....	2
Propagation of bulbils.....	2
Scaling vs. bulbil propagation.....	2
Environmental effects	
On bulbil growth.....	3
On flowering.....	4
Thesis objectives.....	4
Literature cited.....	6
MANUSCRIPT	
Some effects of photoperiod and temperature in propagating <u>Lilium tigrinum</u> bulbils.	
Abstract.....	7
Introduction.....	8
Materials and Methods.....	10
Results and Discussion.....	13
Literature Cited.....	21
APPENDIX.....	22
ACKNOWLEDGEMENTS.....	30
VITA.....	31
THESIS ABSTRACT.....	32

INTRODUCTION

Although the traditional Lilium grown and sold in the United States today is the Easter lily, a number of other types of suitable forcing lilies exist. Since early in the 1950's numerous new cultivars of colored lilies for home gardens have increased in popularity. Some of these new cultivars have a potential to be forced as pot plants and grown for year round production. These colored lilies present a wide range of flower types, leaf shapes, color combinations, and other features that have not previously been available to the public.

If the demand for colored lilies as pot plants should increase, then mass production of forcing size bulbs could be a problem. At the present time, the most efficient and commonly used propagation method is scaling. Aerial bulbil propagation may be used as an alternative to this method.

Aerial bulbils have not been used commercially for several reasons. Under normal conditions not all lilies form aerial bulbils. Secondly, the time required to produce a flowering bulb from a bulbil takes several years. In addition, the percentage of bulbs that ultimately flower from bulbils is small because of disease and other problems. Overcoming these last two problems could be done by growing earlier stages of these lilies in the greenhouse, and controlling growth and development rates by environmental regulation.

LITERATURE REVIEW

Aerial bulbils are naturally produced by Lilium bulbiferum, L. myriophyllum, L. sargentiae, L. sulphreum and L. tigrinum (3, 6) and the hybrids of L. sargentiae and L. tigrinum (1). Several other species produce aerial bulbils under certain conditions but these conditions may not be the best for over-all growth of the rest of the plant.

Feldmaier (4) and Grove (7) reported that the propagation of aerial bulbils is a good method for rapidly increasing bulk number of a new lily cultivar to be released onto the market. Mathematically, it would be better to select clones which propagate by aerial bulbils. In the first year many produce bulbils and in the second year some will bloom if the proper size has been reached.

In the species of lilies that produce aerial bulbils the average number of leaves per plant is approximately one hundred. The number of bulbils varies among species but it is between two to four per leaf axil. If all the aerial bulbils grew, the number of plants would be several hundred.

Bulbils propagated from aerial portions take longer to bloom as compared to scale bulblets. Little work has been done to increase the rate of growth of bulbs.

Griffiths (6) found that in scaling the average number of bulblets obtainable on one scale is between two to five, depending on the species of lily. It is possible to induce more bulblets per scale but as the number is increased the size of the bulblets is decreased. The average number of scales per bulb that can be expected is about

fifty, varying with species of lily. Thus over 100 bulblets may be expected from a single bulb. Removal of all the scales during one year reduces vigor of the mother plant and production the next year.

Griffiths (5) lists L. tigrinum as profusely producing aerial bulbils, but he found that scaling is a more efficient propagation method. He stated that in three or four cases of propagating L. tigrinum by scaling it was better than by aerial bulbils.

The major problems in bulbil propagation are unreliable germination and slow rate of bulb growth. Prior to germination bulbils require a cold treatment in order to induce leaf growth (4, 12). Dean (1) states that in quick germinating types of lily seedlings the bulbs should be given a cold treatment as soon as they have germinated and then allowed to grow. When growth slows another cold treatment should then be given the seedlings. If this is continued for several treatments flowering might occur in the first year.

Aerial bulbils of most cultivars are ready for picking some time just after the flowers begin to fade. This would normally be in late summer months in midwestern states. Although in some late blooming cultivars they may never properly develop due to early frost.

Temperature and humidity have an effect on the development of the bulbils on the mother plant. Feldmaier (4) and Grove (7) have shown that at high temperatures, and either high or low humidity aerial bulbil formation is stimulated. Lillies react differently to high and low humidities. In high humidity the bulbils seem to form without the loss of flower bud count. Whereas, in low humidity flower buds are not formed but aerial bulbils are produced. They also found that the removal

of the flower buds will stimulate aerial bulbils. Also the removal of the mother bulb from the stem will increase production of aerial bulbils.

Most of the work has been done on the forcing of bulbs to bloom. It has been found that to rapidly force an Easter lily, bulbs need several different temperature treatments. There is a critical thermoperiod just after the bulbs have been dug in the late summer. This temperature is 80° F for two weeks (2). Bulbs need 63° F for two to four weeks after potting to remove cold effects and to start rooting, then 35° F to 40° F for five to seven weeks to initiate flower primordia. Then, depending on the time of Easter, it takes between 62° F and 65° F for 90-120 days to force the Easter lily into bloom.

Kohl (9) has reported that light intensity and day length can control height, number of days to flowering, and bud count. These responses to light have largely been thought to be independent of temperature. However, long days will substitute for cool temperature flower initiation requirements (13).

Hartsema (8) stated that flower formation and earliness of flowering are not the only effects of cold temperature. Flowering and lateral bulb development are not favored by the same temperature treatment. Therefore, the importance is shown of determining adequate temperature treatments for different practical purposes.

The objectives of this study were to see if modification of temperature and photoperiod could be used to increase growth of L. tigrinum bulbils. In nature, bulbil growth is stimulated by long photoperiods in the summer months and by cold periods of various durations

during winter months. Combinations of photoperiod and various durations of cold (4.4° C) were reproduced in greenhouse conditions to attempt to rapidly force L. tigrinum bulbils.

Results are presented in manuscript form that will be submitted to the Journal of the American Society for Horticultural Science for possible publication.

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SOME EFFECTS OF PHOTOPERIOD AND TEMPERATURE IN
PROPAGATING LILIUM TIGRINUM BULBILS.¹

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Abstract: Lilium tigrinum aerial bulbils were grown and their response measured to controlled greenhouse light and temperature conditions. Twelve weeks after removal from mother plants, the leaf, root, and bulbil growth of L. tigrinum bulbils were similar in photoperiods of 9, 14, and 18 hrs. However, bulbil germination, leaf number, and length were significantly increased by six weeks of 4.4° C. Bulbils, eight months after removal from mother plants, grown in alternating 4.4° C and 16° C for 3, 6, 12, 24, and 48 day periods produced significantly more leaf number, root length, and germination than did either the outdoor grown or the non-cold treated bulbils. Significantly larger bulbils occurred in the 48 day duration treatment than in the 3, 6, 12, and 24 day treatments.

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INTRODUCTION

Aerial bulbils may have potential as a successful method of propagating certain types of lilies. Emsweller (3) and Feldmaier (4) indicate that bulbil propagation would be the best way of producing Lilium tigrinum, L. sargentiae, and other species.

Hartsema (5) has stated that bulbil growth is influenced by temperature requirements at critical developmental periods to insure that certain physiological processes occur. These optimum temperature requirements, the critical periods, and their sequences may be different for flowering and vegetative growth. A bulb will flower as soon as it reaches a certain size provided that it has been given proper temperature treatment.

In work with small bulbs it has been shown that a cold period of two to three months at 2-8° C is needed in order for top growth to appear (4). The use of cold treatments is not uncommon on other small bulbs as Payne (7) and Dean (1) also show where they have used cold to obtain growth. Dean indicates that as soon as seedlings have germinated a cold treatment should be given them. After they are taken out of the cold and grown for a while they will reach a point where their activity stops. They should then be given another cold treatment. If this is done continually, blooming might be induced the first year. Durkin and Hill (2) have shown that three weeks of 50° F on either side of two weeks of 70° F will increase leaf and flower numbers in 'Ace' lily bulbs, while the flowering date is delayed.

Kohl (6) states that bud count and height are influenced by

long photoperiods in Easter lilies, and that photoperiod is largely independent of temperature over the range of 50° to 80° F. Although in the process of flowering it has been shown that long days can be substituted for cool treatment in Easter lilies (8).

In nature, bulbils are exposed and may respond to alternating cold periods and photoperiod changes. This research examined greenhouse controlled photoperiods and temperatures and their effect on leaf and root growth, bulb size, and germination in L. tigrinum as a means of increasing rate of bulbil growth.

MATERIALS AND METHODS

Photoperiod and temperature study: *L. tigrinum* bulbils approximately .5 x 1.0 cm were picked on May 17, 1972 from greenhouse forced flower stems. Bulbils were stored at 15° C until they were randomly selected and planted in both clay and peat 7.5 cm pots on June 6, 1972. A potting mix of equal parts of soil, peat, and haydite was used. Benomyl was applied as a soil drench after planting. Bulbils were grown pot to pot in wooden flats and watered daily with approximately 100 ppm N from a 20-8.8-16.6 fertilizer.

Each experimental unit consisted of 24 bulbils. Greenhouse photoperiod treatments were (a) 9 hrs (shade cloth pulled from 4 p.m. to 7 a.m.), (b) 14 hrs natural light, and (c) 18 hrs (1000 ft-c supplemented by Sylvania Gro-Lux fluorescent light from 6 p.m. to 12 a.m. Four temperature treatments were (1) 12 wks of 21° C; (2) 6 wks of 4.4° C, 6 weeks of 21° C; (3) 3 wks of 4.4° C, 9 wks of 21° C; (4) 3 wks of 4.4° C, 3 wks of 21° C, 3 wks of 4.4° C, 3 wks of 21° C. Variation in greenhouse temperature was $21 \pm 10^{\circ}$ C and in the cooler temperature $4.4 \pm .5^{\circ}$ C.

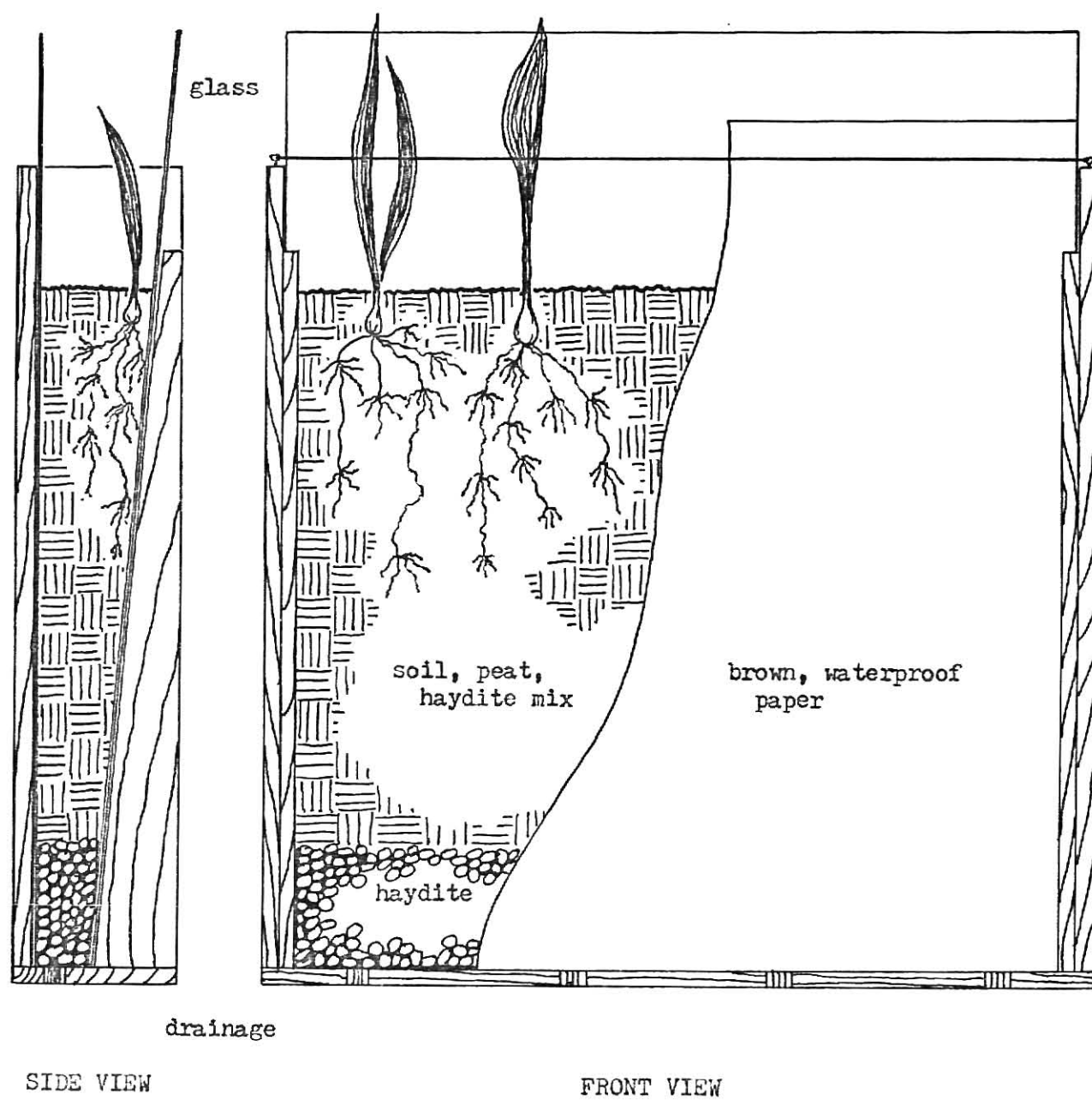
Bulbil data were taken on percentage dormancy and germination, length and number of leaves and roots, and height and width of bulbs after 12 weeks on September 11, 1972.

Alternation of temperature study: *L. tigrinum* bulbils were measured (average width = 1.08 cm), marked as to their previous photoperiod-temperature treatment, and replanted on October 20, 1972. Bulbils were planted in 45 x 45 x 5 cm V-shaped glass lined root chambers (Fig. 1) so that bulb enlargement and root growth could be studied. Brown,

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Fig. 1. Experimental root chamber used to study root and bulb enlargement.



waterproof paper was placed over the glass so that light would not influence root growth. Twelve bulbils were placed in each box in a mix of equal parts of soil, peat, and haydite. Benomyl was applied as a soil drench after planting, then reapplied four weeks later. Bulbils were watered with a dilute fertilizer solution daily in the greenhouse. Comparisons were made at the end of the study with similar aged outdoor grown bulbils planted July 4, 1972.

Experimental root chambers were placed in either 0, 3, 6, 12, 24, or 48 days at 4.4°C . Within each respective time period the treatments were altered between $4.4 \pm .5^{\circ}\text{C}$ and $16 \pm 5^{\circ}\text{C}$ for 96 days. Bulbils were then grown for an additional 30 days at 16°C . There were two replications of each treatment.

In the 16°C treatment, bulbils were grown in the greenhouse under Sylvania Gro-Lux fluorescent bulbs with approximately 1000 ft-c. These were turned on automatically to give 14 to 15 hour photoperiods. No supplemental lighting was supplied bulbils receiving 4.4°C treatment.

Data was taken every three days for germination, root and leaf number and length. At the end of 126 days, data was taken on percentage dormancy and germination, length and number of leaves and roots, and the height and width of the bulbs.

Statistical analysis of data was determined using a two-way analysis of variance for the first experiment and a one-way analysis of variance for the second.

RESULTS AND DISCUSSION

Photoperiod

Lilium tigrinum bulbils produced similar leaf number and length, root number and length in 9, 14, and 18 hour photoperiods (Table 1). This lack of bulbil response to long photoperiods may be some preconditioned factor caused by the mother plant or some dormancy factor in the bulbil. Kohl (6) has reported that for maximum bulb growth of L. longiflorum, long photoperiods are necessary to assure a high dry weight gain per day. Wilkins (8) has reported that long photoperiods can be substituted for all or part of the cool temperature periods thought necessary to hasten flowering of L. longiflorum. Long photoperiods and cold temperatures were not substitutable for promoting bulbil growth in L. tigrinum. Thus indicating that growth and flowering responses are apparently controlled by different biochemical or physiological reactions.

Temperature

Leaf growth: As shown in Table 2, leaf number and length were significantly increased with 6 weeks of 4.4° C temperatures, as compared with bulbils grown at 21° C. Leaves of bulbils grown in the alternation of three week periods of 4.4° C and 21° C were similar as those in six weeks of continuous 4.4° C.

In the alternating temperature study, as shown in Table 3, the number of leaves was significantly increased in all greenhouse treatments as compared to outdoor grown bulbs. Leaf length was also

Table 1. *L. tigrinum* leaf and bulbil growth as affected by photoperiod. Data recorded at the end of 12 weeks in the greenhouse.

Hrs. of light	Leaf		Bulb		Roots	
	No.	Length (cm)	Width (cm)	Height (cm)	No.	Length (cm)
9	1.4a ^z	11.7a ^z	1.0a ^z	.9a ^z	3.8a ^z	12.8a ^z
14	1.5a	10.6a	1.2a	1.0a	4.3a	13.7a
18	1.5a	10.1a	1.1a	1.0a	4.8a	13.7a

^z Values followed by the same letter in any one column are not statistically different at the 5% level. (Duncan's multiple range test)

Table 2. L. tigrinum bulbil germination, leaf and root growth as affected by 4.4° C or 21° C temperatures. Data recorded at the end of the 12 week experimental period.

Total wks of 4.4° C	Total wks of 21° C	Leaf		Root		Germination %
		No.	Maximum Length (cm)	No.	Maximum Length (cm)	
0	12	1.5c ^y	8.6b ^y	5.0a ^y	13.6a ^y	56b ^y
3	9	1.3bc	11.7a	4.1a	13.7a	82a
6	6	1.6ab	12.1a	4.0a	13.0a	86a
6 ^z	6 ^z	1.7a	12.2a	4.2a	13.2a	92a

y Values followed by the same letter in one column are not statistically different at the 5% level. (Duncan's multiple range test)

z Treatment was alternated 3 wks 4.4° C, 3 wks 21° C, 3 wks 4.4° C, 3 wks 21° C.

Table 3. *L. tigrinum* bulbil germination, leaf and root growth as affected by alternating temperatures. Data recorded at the end of 126 days.

Duration of alternating 4.4 or 16°C treatment (days)	Leaf		Root		Germination %
	No.	Length (cm)	No.	Length (cm)	
3 ^x	1.2a ^y	21.2a ^y	5.5ab ^y	21.2b ^y	95
6	1.2a	20.0a	6.6a	20.0b	100
12	1.2a	25.9a	6.6a	25.9a	100
24	1.3a	19.5a	6.7a	19.5b	100
48	1.2a	26.5a	7.1a	26.5a	100
Greenhouse 16° C	1.2a	9.7b	4.6b	9.6c	20
Natural outdoor temp.	1.0b	6.9b	3.5b	2.1d	34

x All treatments received a total of 48 days at 4.4° C or 21° C.

y Values followed by the same letter in one column are not statistically different at the 5% level. (Duncan's multiple range test)

z Treatment grown outdoors from July 4, 1972 to March 17, 1973.

significantly increased in all 4.4°C treated bulbs. However, total duration of the alternating 4.4°C and 16°C temperature periods is the critical factor in determining leaf length. Etiolation in the dark, 4.4°C thermoperiods was not considered to be a factor in increasing leaf length, as some bulbils did not germinate until after receiving this treatment.

Root growth: As shown in Table 2, root growth was similar in all temperature treatments. However, in Table 3, when bulbils were slightly more mature, root numbers and length were significantly increased by 4.4°C treatments. The glass boxes designed to study the bulbils and their root development were inadequate. The detection of bulbil root development was reduced by the cold glass and the media. More work needs to be done on how to measure root growth in cold temperatures. Also, the lily root system is fibrous and hard to study with this method.

Germination: Bulbil germination was significantly increased with 3 to 6 weeks of 4.4°C (Table 2). The non-germinating bulbils in the 21°C greenhouse were considered to be dormant. As shown in Table 3, germination of bulbils was significantly increased in all of the 48 day (4.4°C) treatments as compared to the 16°C treatment. The bulbs grown outdoors germinated better than those in the 16°C greenhouse treatments. Feldmaier (4) has reported that for top growth to occur it takes from two to three months of cold treatment. In contrast, L. tigrinum bulbils respond with as little as three weeks but obtain maximum germination with 48 days of 4.4°C temperatures.

Little germination occurred in the 48 day treated bulbils until

about 10 days after they were placed in the 16° C greenhouse. Then the 48 day treated bulbils germinated uniformly within a week. The bulbils in other treatments as they passed about 40 days of accumulated cold began to germinate. The 3 day alternated temperature bulbils were the last to germinate 100%.

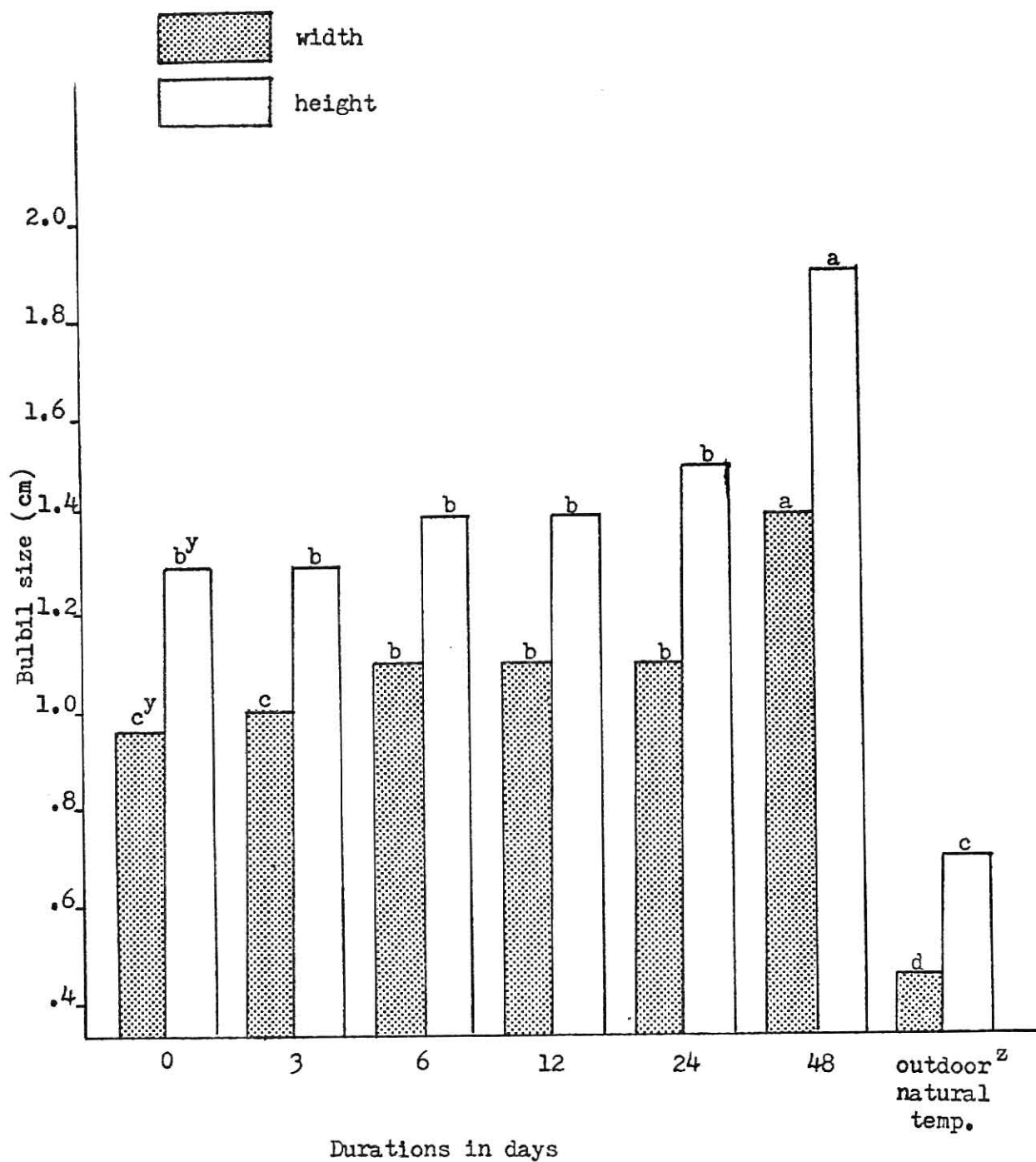
Bulb size: The bulbils receiving the 48 days of continuous 4.4° C then 21° C were significantly larger than the bulbils in the 3, 6, 12, and 24 days alternating temperature treatments (Fig. 2). The greenhouse grown bulbils were significantly larger than the outdoor grown bulbils. Once bulbils have received 4.4° C, higher temperatures (16° C) are needed to increase growth of the bulb.

Over a 30 week time period (Fig. 3) it can be seen that two 6-7 week continuous 4.4° C treatments doubled bulbil size as compared to non-cold treated bulbils, but tripled growth over outdoor grown bulbs. As Dean (1) indicated, bulbils can be kept growing provided they are given correct thermoperiods. In L. tigrinum, 40-48 days of 4.4° C followed by about 80 days of 16° C will keep bulbils actively growing.

The biochemical reaction which causes the difference in the sizes of the bulbs might be one that converts starches to sugars. Hartsema (5) states that there is an increase of sugars in tulips when stored at low temperatures. Thus, when continuously alternating from cold to warm temperatures, bulbs never are able to accumulate starch.

In summary, for maximum bulb growth, continuous 4.4° C for approximately 48 days was found to significantly increase bulb size over alternating temperature treatments. More research should be done to find the optimal length and degrees of constant temperature for best bulbil growth.

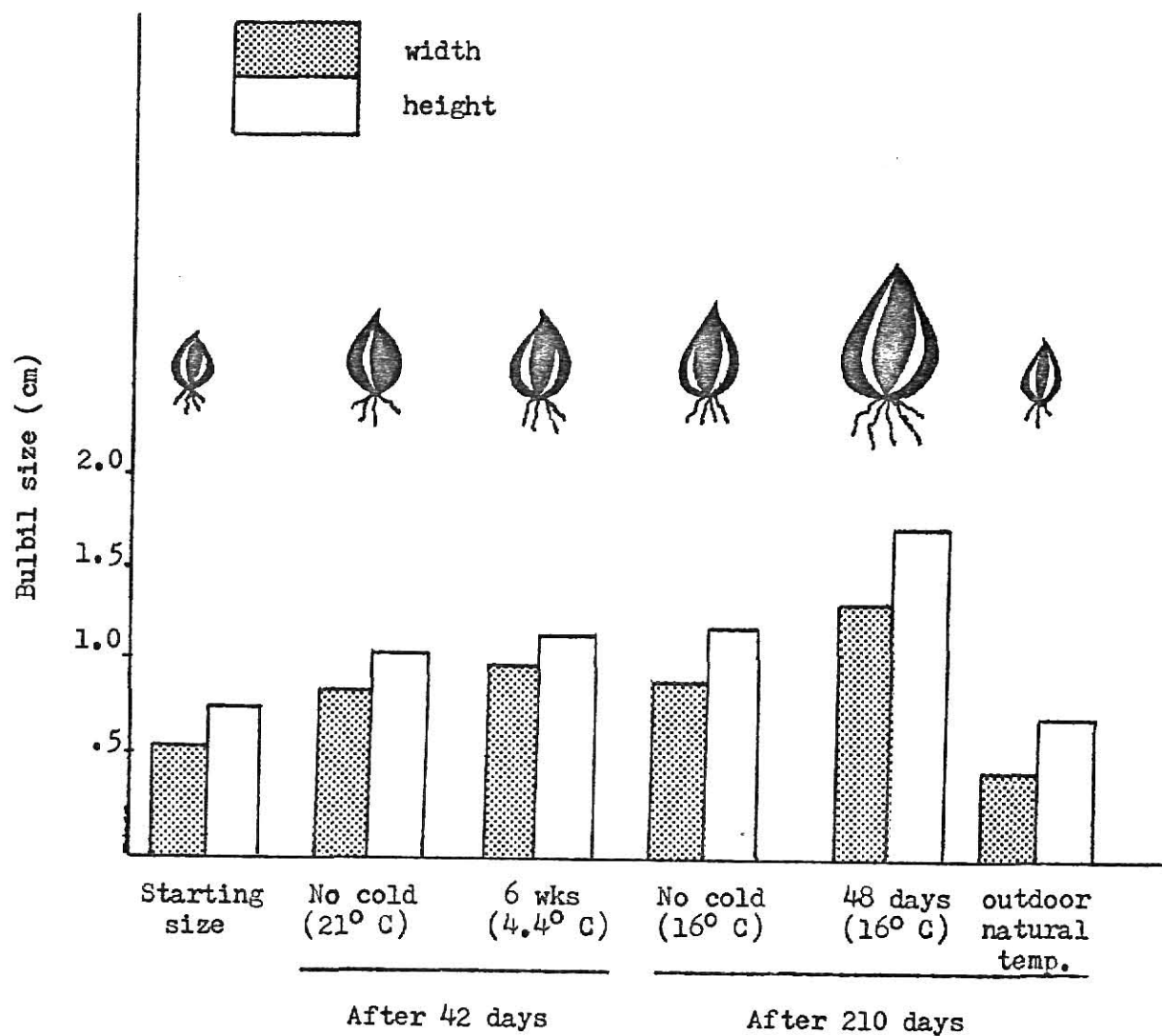
Fig. 2. Bulbil width and height of *L. tigrinum* as affected by different durations of alternating temperature of 4.4°C and 16°C .



y Difference in letter subscript indicates significance at the 5% level (Duncan's multiple range test)

z Treatment grown outdoors from July 4, 1972 to March 17, 1973 did not receive standard 48 days of cold.

Fig. 3. Bulbil width and height of *L. tigrinum* over a 30 week period.



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APPENDIX

Experimental Procedures

Bulbils: Bulbils of L. tigrinum were picked on May 17, 1972 from plants grown in the Horticulture-Forestry greenhouse. These bulbils were easily removed from leaf axils and some had small roots that had grown through the leaves on the mother plants. Bulbils were then stored at 16° C until planted.

Bulbils of Lamb's yellow hybrid were picked in September, 1972 from plants grown in the back yard of Mrs. Reginald Painter, 3006 Claflin Road, Manhattan, Kansas. These bulbils were small and some were hard to remove from the stems. When studies were run, low germination and bulbil survival occurred. The determination of when these bulbs are 'ripe' and ready to pick could also increase the germination percentage and growth rates.

Experimental root chambers: Two lites of B grade, 26 oz. glass were vertically supported to provide an area of 40 x 40 cm. The top width was 5 cm with the bottom being 2 cm giving the box a V-like structure. A piece of brown, waterproof paper was placed on the outside of the glass so that light would not affect growth of the roots. It was hard to see the roots as they grew along the side of the glass, and impossible to see the bulbils (Plate 1).

Another problem was that the roots did not seem to grow along the glass especially in the short temperature duration treatments. Roots seemed to hit the cold glass then turn back into the soil. Roots reappeared on the glass pane after the unit was placed in warmer temperatures.

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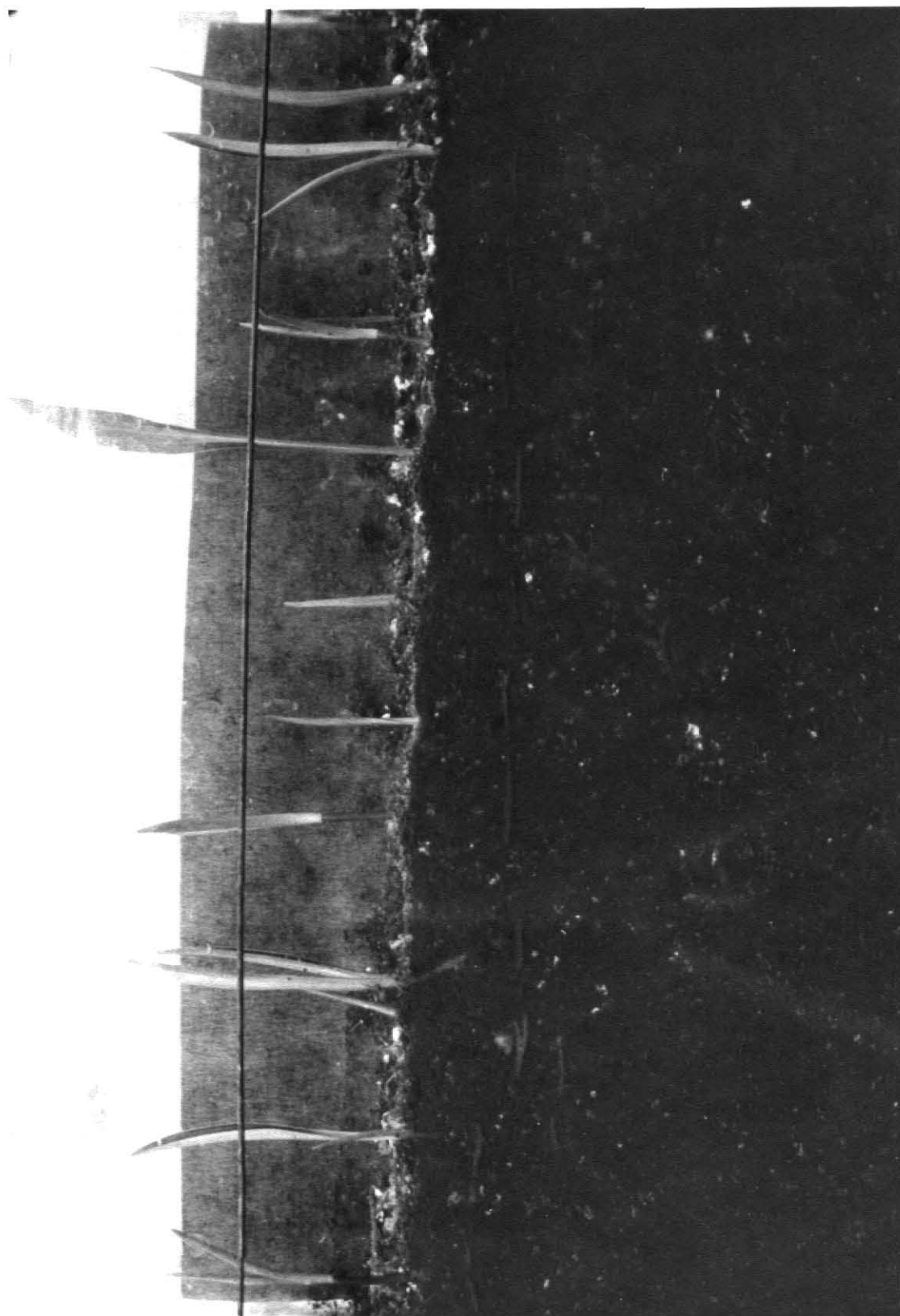
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Plate 1. Experimental root chamber showing twelve bulbils.

A few bulbils and roots can be seen but most
are not visible. The bulbils in the photograph
are approximately actual size.

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In Plate 2, the fibrous root system of L. tigrinum is shown. This root system was a problem as many root tips were visible at one time and hard to determine the maximum root growth at any given time.

Statistical Analysis: A typical analysis of variance keyout for leaf number in the photoperiod-temperature study is found in Table 1. The analysis of variance for germination percentage in the alternating temperature study is found in Table 2.

Plate 2. Bulbil of Lilium tigrinum showing fibrous
root system. Enlargement is approximately
twice actual size.

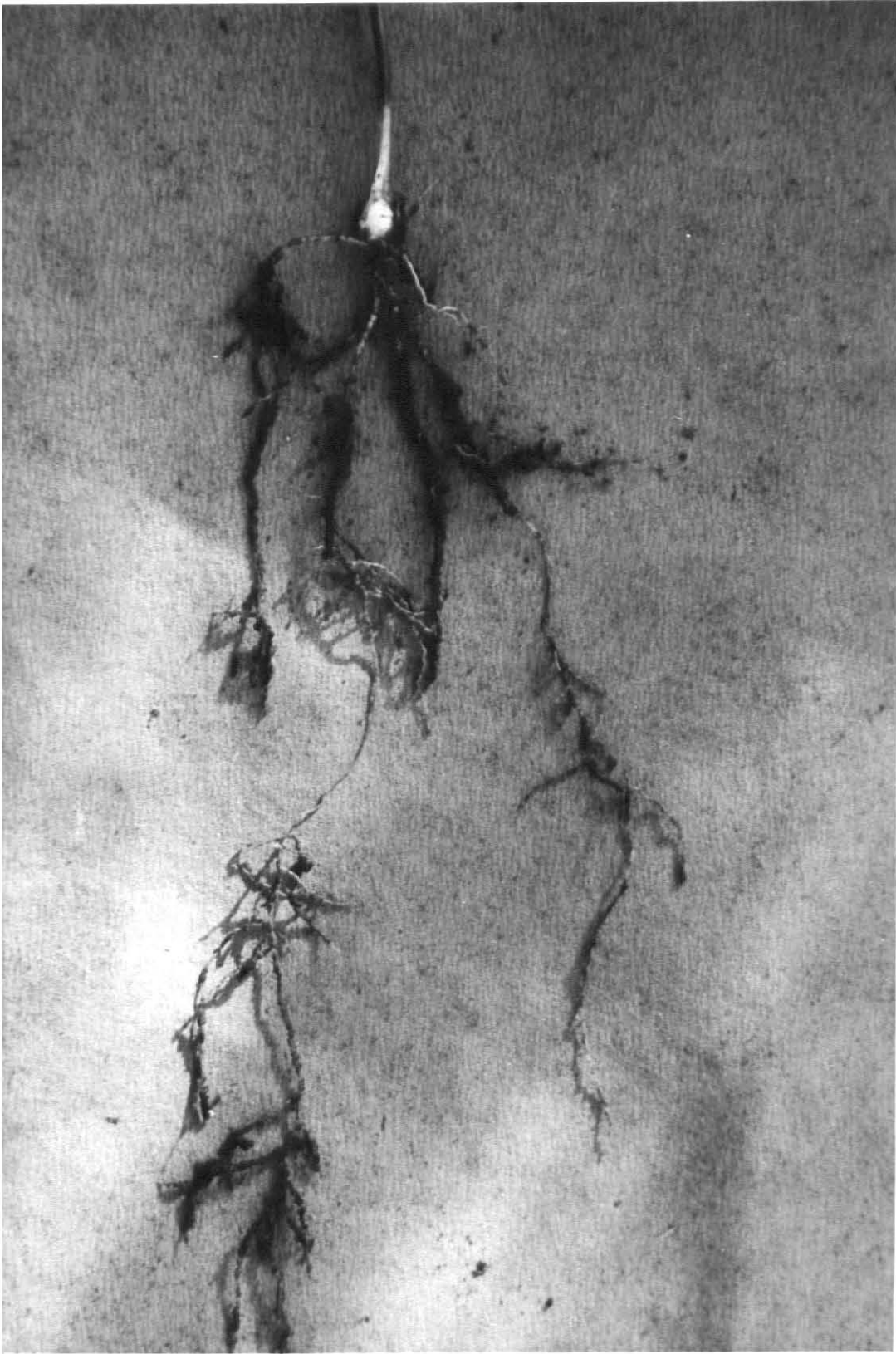


Table 1. Analysis of variance of percentage germination
for photoperiod-temperature study.

<u>Source of variance</u>	<u>Degrees of freedom</u>	<u>Mean square</u>	<u>F value</u>
Photoperiod	2	195	.82
Temperature	3	1476	6.23*
Photoperiod x temp	6	71	.30
Pots	1	1349	5.69*
Error	11	236	
Total	23		

* Significant at the 5% level.

Table 2. Analysis of variance of leaf number, alternating temperature study.

<u>Source of variance</u>	<u>Degrees of freedom</u>	<u>Mean square</u>	<u>F value</u>
Treatments	7	55	5.08*
Error	164	10	
Total	171		

* Significant at the 5% level.

ACKNOWLEDGEMENTS

The author wishes to acknowledge Dr. Richard Mattson for the assistance and encouragement given throughout the course of study. Sincere appreciation is extended to Dr. Charles Hall, Professor of Horticulture, for help given during my study at Kansas State University.

Special thanks are extended to Dr. Ronald Campbell, Head of the Department of Horticulture and Forestry, and Dr. Cecil Nickell, Assistant Professor of Agronomy for suggestions. Appreciation is extended to other members of the Horticulture and Forestry Department for their help and suggestions.

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SOME EFFECTS OF PHOTOPERIOD AND ALTERNATING TEMPERATURE
ON ROOT AND SHOOT DEVELOPMENT OF LILIUM TIGRINUM BULBILS

by

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B. S., Fort Hays Kansas State College, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture and Forestry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1973

The greenhouse propagation of Lilium tigrinum aerial bulbils was investigated as influenced by controlled photoperiod and temperature treatments. Germination, leaf, root and bulb growth of aerial bulbils were measured over a 10 month period.

Aerial bulbils were harvested from mother plants and grown in 9, 14, or 18 hour photoperiods. Bulbil growth was similar in all day-lengths.

L. tigrinum bulbils receiving cold (4.4°C) treatments produced significantly larger leaves, roots, and percentage germination than did those bulbils grown outdoors or in a 16°C greenhouse. Bulbils grown continually in 4.4°C for 48 days were significantly larger than those grown in 4.4°C for 3, 6, 12, or 24 day intervals.