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# Fundamental Studies on the Double Nosed Bulb Formation of Easter Lily

By

# Tadashi Kubo

Department of Horticulture, College of Agriculture

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## 1. Introduction

In the northern climates of Japan, the quality of bulbs of *Lilium longiflorum* suffers from the following two defects, inadequate increment of the size and the occurrence of the so-called double nosed bulbs. Such bulbs occur, however, less frequently in warmer regions as in Saitama and Kagoshima prefectures. Growers believe that easter lily bulbs will produce the so-called double nosed bulbs after they get more than 7 inches circumference of the bulb or the older bulbs will split naturally. As a matter of fact it seems that they appear in older or larger bulbs even under the same meteorological conditions.

Irmisch, T. (1890) published some information on lilies also in his book "Zur Morphologie der monokotylischen Knollen und Zwiebelgewaechse". The research group of Waageningen (Blaauw, A. H., Hartsema, A. M., Huisman, E., Luyten, I., Mulder, R. and Zweede, A. K. etc.) reported elaborate morphological observations on the growth behavior in Hyacinthus, Narcissus, Tulipa and Convallaria etcetera. Pfeiffer, N.E. (1935) reported the fundamental information on the morphology of new bud and flower formation. Recent studies on easter lily, in the field of horticulture, have been concentrated on the cut flower production or on the bulb production for cut flower. Studies of Brierley, P. (1941), Brierley, P. & A. H. Curtis (1941), Emsweller, S. L. & R. L. Pryor (1943), Post, K. (1941) and Stuart, N.W. (1943-) on forcing reported various results using divergent bulbs of the individually different growth period or in the different conditions of the air or the soil in each case, or also using bulbs grown in the different conditions. For these reasons it is quite important that every data should have enough records on their growth periods and the meteorological conditions at least at the time of digging out the bulbs and after the digging. For, under these conditions also, there is no interruption of growth in the bulb. Although a number of ornamental monocotyledons have been the subject of study, sometimes with practical application in forcing, the accidental production of split bulbs in easter lily has not received attention before previous papers published in 1949.

To elucidate the condition of the accidental formation of the double nosed bulbs in northern climates, the various phases of the development of normal and split bulbs were studied from the morphological and physiological point of view.

### 2. Material and methods

Two varieties, *Lilium longiflorum var. insulare* and *Lilium longiflorum var. takesima*, were used as material. The grower's practices in Saitama prefecture were employed in this experimental cultivation. The material and methods in this studies are as follows.

Morphological observations of the bulb: Used *var. takesima* bulbs of Saitama prefecture. With double nosed bulbs of various types bulbs of Hokkaido were applied occasionally.

Behavior of the scale leaf development: Used *var. insulare*. The distribution of old and new scale leaves on the transverse section of bulbs at periods (cf. Plate 1–1 to 8) were pursued on occasion.

Growth of tops and double nosed bulb: Used *var. insulare*. The height and diameter of stems, weight of the mother bulbs and the increase of bulb weight were measured and compared with the occurrence of double nosing.

Development of the bulb and double nosed bulb: Used var. insulare. They were weighed before planting and after digging up and compared with each other.

Diseases and double nosed bulb: Used var. insulare. The condition of diseases and their occurrence were recorded and compared with each other.

Depth of planting and double nosed bulb: Used var. insulare and var. takesima. After weighing bulbs, they were planted about 2 cm. and 8 to 10 cm. deep, and the differences between these two were examined on the occurrence of double nosing.

Injury of growth and double nosed bulb: Used var. insulare. Some treatments as injuries to leaves, stems and roots were applied in the manner mentioned below.

Occurrence of the double nosed bulb in Hokkaido: Used var. takesima produced in Saitama prefecture and in Hokkaido. The grower's practices in both locations were employed in each place.

The incentive and process of double nosed bulb formation: Used two varieties mentioned above. The region and the period of the new bud in the bulb were observed anatomically, and studied on the periodical development of the bulb.

Plastic substances in the bulb: To substantiate the morphological observations more precisely, reserve substances such as starch, reducing sugar, non-reducing sugar, soluble nitrogen and protein nitrogen were measured by chemical analyses of bulbs from various lots under the various conditions between April and October. Analyses were made on small bulbs (3–5 gm. at the time of planting) and larger ones (15–20 gm.) using 5 wedge-shaped cut pieces in small bulb analyses and 3 pieces in larger bulb analyses. The methods used in obtaining juice, preparing the juice for the analyses, and analitical procedure in details are the same as those described in the previous paper.

Influence of the external factors: The influence of temperature, moisture, pressure, hydrogen ion concentration and light were examined. Among these only the temperature treatment, because of its rich effect despite of the dull effect in others, was employed with great care. They were placed in two different cold storages at four or five different stages of three different weight bulbs. And these two different cold storages have divided

into five different treatments. Both varieties were used.

Split bulb formation in the propagation by scale leaf: Used *var. takesima*, 969 scale leaves. They were planted in November, placed in the green house by early May, and outside from the end of May after hardening.

## 3. Experimental results

General observations on the double nosed bulb.

Irregularity of the bulb shape in easter lily depends on various factors, such as soil condition, depth and spacing of the bulb in the soil, and formation of the double nosed bulbs etcetera. Wilson, E. (1925) reported the double nosed bulb in natural, and growers in Japan, especially in the northern climates, have been cautious to minimize the occurrence of double nosed bulbs with poor growth and abnormal shape.

Morphological ovservation of the bulb.

The structure of an easter lily bulb is divided into two broad parts, base axis and scale leaves (Fig. 1). Usually large bulbs bear scale leaves from two seasons. It is clear that during a part of the year two growing axises are present in one bulb, but from the

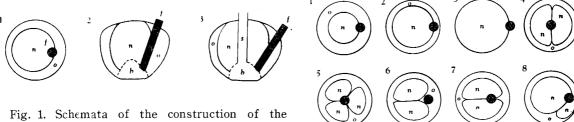


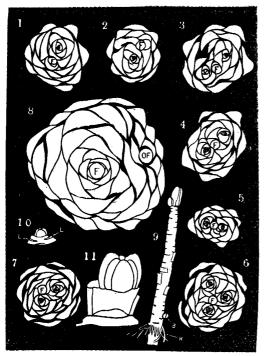
Fig. 1. Schemata of the construction of the easter lily bulb. 1. Cross section of the large bulb in fall. 2. Longitudinal section of that in fall. 3. Longitudinal section of the large bulb in next summer. n-new daughter bulb, o—two-year part, f—old flower axis, b—bulb axis, both of the new daughter bulb and mother bulb, s—flower axis.

Fig. 2. Schemata of the type of the easter lily bulb. n- new bulb which was formed and enlarged from this spring. o—two-year part which was formed last spring and enlarged again this year. Black circlet—old flower axis.

time of the death of this year's flower stalk until shortly after planting only one is present. Speaking of their external shapes, they are divided into five shapes (Fig. 2) as in the followings.

- Type 1. With the old flower stalk near the center (Fig. 2-1)
- Type 2. With the old flower stalk near the outside (Fig. 2-2, Plate 2-8)
- Type 3. With the old flower stalk outside (Fig. 2-3)
- Type 4. Divided into more than two new bulbs of almost equal size (Fig. 2-4 to 7)
- Type 5. Divided into more than two new bulbs of different sizes (Fig. 2-8)

There is no question that bulbs of Type 1 are the best in bulb production. Bulbs of Type 1 have more than treble two-year scale leaves at the outside of the old flower stalk. Most of bulbs one year after planting bulblets are of Type 3. From the difference of the position of new bud formation, the double nosed bulbs are divided into two broad shapes as follows.



#### Plate 1.

- 1. Cross section of the bulb grown in Yoichi, Hokkaido, on June 15. Stem length 20.5 cm. New daughter bulb was hatched. Diameter of the whole bulb was 4.1 cm., that of the new daughter part
- 2. Cross section of the bulb of whole bulb diameter 3.7 cm., new daughter part 1.6 cm. and 1.7 cm. stem. June 15. Yoichi.
- Cross section of the bulb grown in Yoichi, June 10. Stem length 15 cm., diameter of the whole bulb 4.3 cm., that of the new daughter part 0.6 and 0.7 cm. An example of double nosed bulb with less growth.
- Same day, same place. Diameter of the bulb 4.2 cm., that of the new daughter part 0.6 and 1.0 cm., stem 15.3 cm.
- 5. Cross section of the bulb grown in Yoichi, May 26. Stem length 12.8 cm., diameter 3.0 cm., that of the daughter part 0.3 and 0.4 cm. This is an example of the bulb, 2 or 3 inches circumference, at that time.
- Cross section of the bulb grown in Yoichi, on June 10. Stem 18.7 cm., diameter 4.5 cm. Triple nosed bulb. The floral axis of this is shown in the Plate 1-9.
- Same day, same place. Stem length 18 cm., diameter 4.5 cm.
- 8. Cross section of the bulb imported from Saitama on September 26. Stem 0.9 cm. without new
- Floral axis of the bulb of Plate 1-6. Stem roots (R) and bulblets are observed. Flower bud of the bulb on May 2, stem length 4.5 cm. L shows the place of foliage leaf. Height of the flower bud 7.0 mm., diameter of that 9.3 mm. Grown in Yoichi, Hokkaido.
- 11. Flower bud of the bulb on May 24, stem length 10.3 cm. Height of the flower bud 10.1 mm., diameter of that 12.0 mm. Grown in Yoichi, Hokkaido.

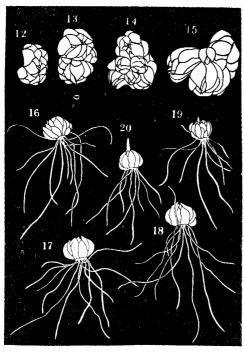


Plate 2.

- 12. Normal bulb which was extincted all of two-year scale leaves.
- 13. Double nosed bulb (Type A-a).
- 14. Double nosed bulb (Type A-c). Three smaller bulbs in the center are the bulblets formed on the basal stem.
- 15. Triple nosed bulb which hold three daughter bulbs formed at one side of the flower axis.
- 16-20. Here showing various stages of the bulb grown in Yoichi on April 2. It seems that bulbs with better growth of stem have less growth of the basal roots.

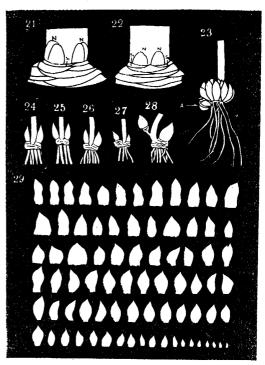


Plate 3

- 21. Two new daughter buds formed on the same axil of the last of scale leaves (S1).
- Two new daughter buds formed on the two different innermost scale leaves  $(S_1S_2)$ .
- Here showing the abnormal formation of bulblet (A) outside.
- 24-28. Here showing the difference of the level of the daughter bulbs.
- 29. Here showing the shapes of scale leaves of the larger bulb. Outer two-year scale leaves have the narrow part. Outer 23 scale leaves seem to be two-year ones.

Type A. Bulbs having two or three equal sized daughter bulbs.

- a. Having the old flower stalk at the center between two daughter bulbs (Fig. 2-4, Plate 1-4, 5, 2-13)
- b. Having the old flower stalk at one side of the two daughter bulbs (Fig. 2-6, 7, Plate 1-3, 7)
- c. Having three or four daughter bulbs (Fig. 2-5, Plate 1-6, 2-15)

Type B. Bulbs having two or three different sized daughter bulbs. (Fig. 2-8, Plate 2-14)

The difference between A and B type may be realized by taking off scale leaves one by one carefully (Plate 3-24 to 28). If the two new buds are formed at the axil of the same scale leaf, it is impossible to find the level difference, but possible to find it by the position relationship between daughter bulbs and the old flower stalk such as in Type A-b. However, bulbs in Type A-a might always have two daughter bulbs formed at the axil of two different scale leaves. There exist some bulbs close to the Type A-a which hold two daughter bulbs formed at both ends of the same scale leaf axil, and which rarely happen in bulb production from small ones. The smaller daughter bulb of the Type B is no doubt a kind of bulblet.

On rare occasions there appears accidental occurrence of a bulblet at the outside of the mother bulb. Sometimes it has its own scale leaf outward. The accidental formation of this kind of bulblet is quite the same, morphologically, as the bud formation in the propagation by scale leaf.

Behavior of scale leaf development.

Large bulb bears two kinds of scale leaves, outer two-year scale leaves of the mother bulb and inner one-year scale leaves of the daughter bulb. But as a matter of fact it is rather difficult to draw an exact line of distinction between two-year and one-year scale leaves (Plate 1–1 to 8, 3–29). Materials were planted at mid September as soon after the bulbs were harvested as possible. Before snow there appeared only the very slow elon-

gation of the basal roots. Then most of them start growth of the stem in the bulb only, but some of them may start gradual growth of the stem outwards. Fluctuation of the weight of bulbs planted in the field are shown in Figure 3. Until the next March slow elongation of the roots and stem continued, and the growth rate remarkably increased from April. Though the decline of the bulb development in weight continued until the beginning of June, the daughter bulb in the

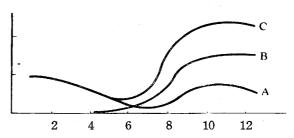


Fig. 3. Relationship between enlargement and exfoliation. A—exfoliation, B—enlargement of the new bud, C—growth of the whole bulb. Abscissa-month, February to December.

mother one began to grow noticeably then. At the beginning of August, after flowering, the decay of outer scale leaves came to an end completely. This process is shown in a

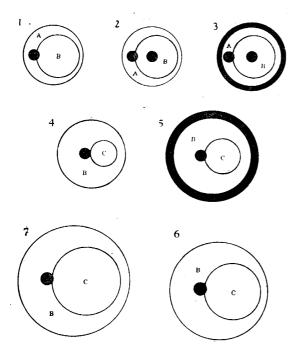


Fig. 4. Schemata of the growth of lily bulb.

1. Construction of the bulb in fall. 2. Next spring stem elongates. 3. Exfoliation of the A, shown as black ring, by spring.

4. Complete exfoliation of the A. 5. Exfoliation of the outer B by early summer.

6 to 7. Without exfoliation enlargement progress only. A, B and C showing different year tissue.

diagram, Figure 4. There exists a great difference in the behavior of the scale leaf development between the smaller bulb or bulblet and the larger one. It comes also from individually different conditions of the bulb and some from external conditions. Scale leaves of the bulblet were lost entirely by the time of harvest. These may be lost up to the end of June in Hokkaido.

Growth of the stem and double nosed bulb.

To make sure the possibility of the relationship between the occurrence of double nosed bulb and the stem growth, all materials were measured in length, diameter of stems and weight increase of the bulbs. With smaller mother bulbs, the difference of the stem growth between bulbs with double nosed daughter bulbs and bulbs with one normal daughter bulb was indicated in the slight inferiority of the stem elongation of the former in early April and at harvest.

With larger ones, there appears no difference between them. Bulbs of the stem height below about 14.3 cm. never produced the double nosed ones. The diameter of the larger ones with double nosed daughter bulbs might be a little thicker than the other's of equal weight.

Size of the bulb and double nosed bulb.

As we see from the reports of Griffiths, D. and Wilson, E. and from grower's experiences that the young bulb does not produce the double nosed bulb, the data showed that bulbs with a weight under 5 gm. did not grow into double nosed ones in Hokkaido, while bulbs with a weight above 10 gm. indicated the mushroom increase in the production of double nosed bulbs. The increase in the weight of whole bulbs with double noses were considerably above that of normal ones.

Diseases and double nosed bulb.

Symptoms and conditions of diseases were recorded with the occurrence of double nosed bulb. The results showed negative possibility to give rise to double nosed bulb caused by diseases. The conditions of diseases, Botrytis blight (Botrytis elliptica), Bacterial disease (Bacillus lilli) and Mosaic disease (Virus), were recorded for two years and bulbs for observations were of 10 to 20 gm. weight at the first planting

Depth in the soil and double nosed bulb.

Bulbs of 1 to 10 gm. weight were planted about 2 cm. and 8 cm. deep and spaced approximately their width in the row. The results showed that small bulbs covered with soil to the depth of about 2 cm. produced abundant double nosed bulbs (40%), regardless of the fact that they usually might produce few double nosed bulbs in the northern climates. In these observations too, bulbs planted about 8 cm. deep produced very few double nosed ones. It intimates that the difference of the external conditions brought about by two different depth might cause the physiological tissue conditions suitable for the initiation of the double nosed bulb. The same kind of phenomena have been reported in tulips and taroes.

Injury to the growth and double nosed bulb.

Some material received injury to tops or roots by cutting them off at various stages to know the possibility of double nosing.

Injury to the top: Bulbs of 5 to 6 gm. weight were picked out as material. All leaves opened were taken off on the 2nd of June. According to the observations of the other bulbs of the same weight, they had about 8 base roots of 10 cm. or more in length, tops of about 6 cm. in height above the soil, about 8 cm. stem length in the soil and about 10 green leaves opened. Not any difference can be noticed on the double nosed bulb production between both the treated and non treated. Judging from this result, the injury to the leaves at this stage does not cause double nosing. And it did not make any difference on the bulblet formation in the soil. The inferiority exists on the increase of bulb weight.

Injury to the roots: Bulbs of 5 to 7 gm. weight were picked out as material. They were replanted as soon as the base roots were cut off with the remains 2 cm. long. Five treatments were carried out at five different periods. Conditions of the material dug up at each treatment are mentioned below. On the 15th of April they had 1 to 7 roots of 2 to 6 cm. in length and tops below 2 cm. long which were still in the soil. On the 30th of the same month and the 9th of May, 2 to 6 cm. tops and 2 to 8 roots of 3 to 9 cm. long. On the 22nd of May, 5 to 9 cm. tops, some stem roots and 5 to 10 roots of 6 to 15 cm. long. On the 2nd of June, 7 to 13 cm. tops, 7 to 10 roots of 7 to 18 cm. long. All bulbs treated on the 30th of April and 9th of May were double nosed, while nearly a half of the bulbs were double nosed by the treatment of the 15th of April. The result showed that this kind of injury from about mid April to mid May was quite incentive to produce the double nosed bulb. The effectual period like this might be more or less shifted according to the different growth progress of variously sized bulbs.

Low temperature, high moisture and darkness after certain stem elongation: Materials of about 5 gm. weight were planted in the test tubes (5 cm. diameter, 25 cm. height and perforated bottom) and these test tubes were kept up in the soil. They were kept in the wet and low temperature (3° to 5°C) room for 14 days after about 5 cm. stem length. Before and after the low temperature they were maintained in the medium temperature, 10° to 15°C, for a few days. About 10 days after bringing them back to the field some

bulblets on the lower axils above the soil were observed with the unaided eyes. The result indicates that the above condition with the low temperature and high moisture produces a lot of accidental new bud in the bulb and on the lower stem axils, while usually they do not produce double nosed bulbs and bulblets in that way. The condition with only high moisture, without low temperature, did not produce double nosed bulbs like that, but produced some bulblets mostly under the soil. Some of materials kept in the dark place for 14 days also, on the other hand, did not produce double nosed bulbs.

Double nosed bulbs in Hokkaido.

The percentage of the production of the double nosed bulb in Yoichi, Hokkaido and Hanazono, Saitama prefecture were compared under the same cultivation, using Hokkaido and Saitama bulbs. The results showed that the Saitama way of bulb production applied in Hokkaido seemed to produce much of accidental double nosed bulbs. The increase of size also was not enough in this northern climates. Therefore it is necessary to search for the suitable method of bulb production in the northern climates, because of the different and unavailable growing stage and time lag, and regulating the cultivation method and the behavior of bulb development.

Process and incentive of the double nosed bulb formation.

As mentioned above there exist some varieties of the split bulbs caused by different positions of daughter bulbs on the axils of the innermost scale leaves. Pfeiffer (1935) reported that the new bud which is to the function in making the following year's floral axis arised in the axil of the last of the scale leaves on the bulb axis; occasionally there were two buds in two leaf axils and the bud was diametrically opposite or at one side with relation to the position of the old floral axis. But no attempt was made to follow the formation and development of the double nosed bulb in detail.

Position and time of the new bud formation.

Pfeiffer (1935) reported that the earliest stage seen in variety giganteum was a prominence of meristematic tissue seen in dissections of month-old plants of December planting and 17-days plants of April planting. Maekawa and Myodo (1948) and Okada (1950) reported the new bud formation in Lilium speciosum f. rubrum and Lilium Hansoni, and in Lilium longiflorum. Judging from the fact that there was no particular difference of tissues in the leaf axil, it is impossible to find where the premodium is in the axil before the meristematic tissues arise (Plate 4-30). The earliest stage seen as a prominence of meristematic tissue was observed in cross sections of the leaf axils. The shoots for this year's flowering, at these times, represented the stem length of about 2 cm. from the top of the bulb (Plate 5-35 to 38). This new bud formation is exogeneous as in scale leaf propagation. The new bud initiation in the bulb, morphologically, is a little different from the usual axilliary bud or latent bud, and on the physiological point of view it is also different from the adventitious bud. According to the process of the new bud formation in the axil of elongated stem or in the axil of the last of scale leaves on the bulb axis, it is rather close to the case of the so-called collateral bud or accessary bud which

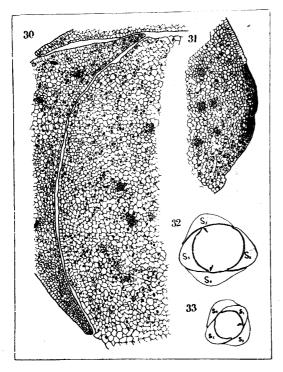


Plate 4.

- 30. Cross section of the axil of the last of scale leaves. The position is shown in 32 of Plate 4.
- 31. Here showing the initiation of the new bud in the axil of the last of scale leaves. The first leaf on the prominence is already observed. The position is shown in 33 of Plate 4.

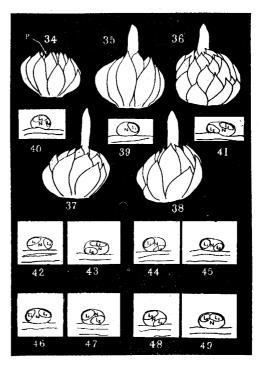


Plate 5.

- 34-38. Here showing the bulbs on October 20th which were imported from Saitama prefecture. They had the new bud prominence already, except for the bulb of 34.
- 40-49. Here showing various positions of the first last  $(L_1)$  and the second one  $(L_2)$  formed on the new bud prominence (N).

holds more than two buds in one axil, perpendicularly or horizontally. Either way they act in their own way in each case. Some produce just one new bud on an axil, some others may produce two new buds on the same axil and on rare occasions more than two bulblets may arise on the same leaf axil. Three new buds on one axil in the bulb were not observed, while observed on the lower elongated stem axils. The position of the first leaf on the new bud looks like random (Plate 5–39 to 49). But generally speaking it follows the principle of the so-called "adossiertes Vorblatt" (back to back bract) formation of axilliary bud in monocotyledons.

In larger bulbs, there appeared generally three innermost axils with fairly equal

| Table 1. | Position i | in ' | the | axil | of | new | daughter | bud | tormed | ın | the | mother | bulb. |
|----------|------------|------|-----|------|----|-----|----------|-----|--------|----|-----|--------|-------|
|----------|------------|------|-----|------|----|-----|----------|-----|--------|----|-----|--------|-------|

| Bulb weight of materials |      | Left   | part of th | ne axil | Middle | part of t | he axil | Right part of the axil |       |    |  |
|--------------------------|------|--------|------------|---------|--------|-----------|---------|------------------------|-------|----|--|
|                          | Last | Second | Third      | Last    | Second | Third     | Last    | Second                 | Third |    |  |
| Bulblet                  | 56   | 12     | 5          | 2       | 15     | 7 .       | 0       | 11                     | 3     | 0  |  |
| 15-20                    | 89   | 18     | 18         | 13      | 21     | 15        | 7       | 12                     | 10    | 15 |  |
| 3035                     | 39   | 7      | 4          | 5       | 6      | 6         | 5       | 8                      | 8     | 7  |  |
| 40-50                    | 13   | 3      | 2          | 2       | . 1    | 2         | 1       | 4                      | 2     | 3  |  |
| Total                    | _    | 40     | 29         | 22      | 43     | 30        | 13      | 35                     | 23    | 25 |  |
| Sum total                | 197  |        | 91         |         |        | 86        |         |                        | 83    |    |  |

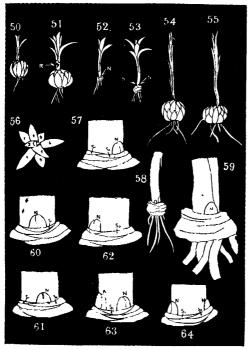


Plate 6.

50-53. Here showing two bulbs of the shallow planting, on April 20th. Stem roots, base roots and the new bud are observed.

54-55. Here showing the other two bulbs of usual planting on May 8th.

56. Here showing the phyllotaxis of easter lily.

57-64. Here showing various positions of the new bud on the axil of the innermost scale leaves. 57—New bud (N) on the center of the axil of the second scale leaf (S<sub>2</sub>). 59—New bud outside of the last scale leaf (S<sub>1</sub>), often seen in bulblets. 60—On the center of (S<sub>1</sub>). 61—On the left side of (S<sub>1</sub>). 62—On the right side of (S<sub>1</sub>). 63—Two new buds on the both side of (S<sub>1</sub>). 64—Two new buds on the center of (S<sub>1</sub>) and (S<sub>2</sub>).

chance for the new bud initiation as seen in Table 1, while in smaller bulbs most of the new bud formations took place in the last two scale leaves on the bulb axis. As it was found that, occasionally, more than one bulblet arose in one axil, or that there existed no rule for the certain position of new bud formation in the leaf axil of the elongated stem, the new buds in the bulb also were formed on the uncertain position in the axils of the innermost scale leaves as shown in Table 1 and in Plate 3-21, 22, 4-30 to 34, 6-57 to 64, 7-65 to 67. Other axils in the bulb except these three had no chance to give rise to the new bud formation. The new bud initiation in the easter lily bulb, for these reason, is far different from the usual phenomena in dormant or latent bud with meristematic tissues. To show the time of new bud formation in the bulb it is necessary that they are indicated by shoot growth having records of temperature conditions because of the very uneven growth. In Hokkaido new bud initiations appeared between November and April. And it represents a stem length of 1 to 2.5 cm. from top of the bulb. But some of the new bud in the bulblets with about 0.8 cm. stem length already had new bud initiations.

Double nosed bulb formation.

The double nosed bulb for mation of 39 bulbs, 20 to 30 gm., of both varieties were observed in the middle of April. Three innermost axils mentioned above, strictly speaking, have the difference of the level. For convenience they are called the innermost, the second and the third. According to the observations of the new bud formation using these two varieties, the new buds arose in these three axils as follows; in

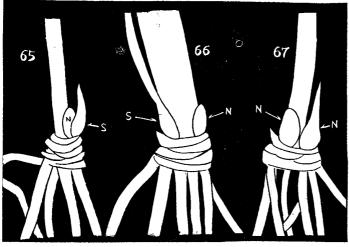


Plate 7.

65-67. Here showing various shapes of new bud and innermost scale leaves in bulblets.

variety takesima, 10 in the innermost, 7 in the second, 6 in the third, 8 in both innermost and second, 4 in both innermost and third, 4 in both second and third, 0 in all these three, and in variety insulare, 11 in the innermost, 6 in the second, 6 in the third, 7 in both innermost and second, 4 in both innermost and third, 4 in both second and third, and 1 in all of these three.

The last scale leaf of the small bulblet may hold the stem completely as seen in Plate 6-59, while most of the stems in bulblets hold the room of the axil of the second scale leaf. In these cases results obtained were quite different from the new bud formation of larger bulb: innermost 42, second 15, innermost-second 2 in 59 bulblets of 3 to 4 gm. of *variety insulare*, innermost 23, second 27 in 50 bulblets of about 2 gm. of *variety insulare*. It rarely happened to give rise to two new buds formed in the same axil of the bulblet.

Periodical development of the bulb.

The life history of the individual bud may be divided into the following six periods, namely embryonic period, leaf forming period, flower forming period, extension period, flowering and fruiting period and exfoliation period in which the outer scales exfoliate. For convenience now, they will be mentioned in the following four subitems.

Embryonic period: The new bud initiation in the bulb, morphologically, is a little different from the usual axilliary bud or latent bud. The embryonic period starts from the beginning of activity in the tissues, first evidence of change, and the prominence of meristematic tissues, and continues to just before the first leaf formation on the prominence. It represents a stem length of about 2 cm. from the top of the bulb. It is on from December to March, mostly in February, in Saitama prefecture. In Hokkaido, however, from November to the beginning of May, mostly in April. The soil temperature then is 1° to 5°C in Saitama prefecture, and 0° to 24°C in Sapporo.

Leaf forming period: This is the period from the first leaf formation on the meristematic prominence to just before the first evidence of change to the flower formation.

It is quite long period, but the development itself is very simple. It is divided 35 into two main parts, scale leaf forming and foliage leaf forming period. 25 Under the temperature of 15° to 18°C, about three days after the beginning of 15 the embryonic period the first leaf arises on the prominence. As a matter 5 of fact the temperature in the soil, in April, in Hokkaido, is not high enough. The average days from the beginning of the embryonic period to the first leaf formation is about 7 days under

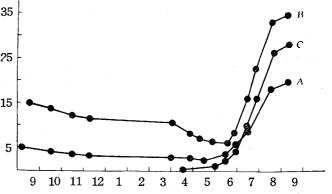


Fig. 5. Periodical change of the bulb weight. A—Small bulb, B—Medium bulb, C—New daughter bulb of the B bulbs. Ordinate-weight in gm., abscissa-month (from September to next September).

the soil temperature 2° to 13°C in Hokkaido. Fluctuation of the weight of the bulbs planted is shown in Figure 5. At the end of the leaf forming period the formation and enlargement of scale leaves come to an end in September, in Hokkaido, and in early August in Saitama prefecture. Then foliage leaf formation appears. Four kinds of temperature treatment after digging them up resulted in different average numbers of the foliage leaf on a stem; 15 days under 17° to 20°C resulted in 28.5 leaves, 15 days under 22° to 25°C resulted in 37.8 leaves, 15 days under 24° to 28°C resulted in 56.5 leaves, 15 days under 29° to 31°C resulted in 50.2 leaves. Bulbs employed were of *variety insulare*, 25 to 30 gm. bulb weight. The optimal temperature of the foliage leaf formation seems to be comparatively high, 24° to 30°C. This may be why bulbs planted soon after digging them up in late September or early October in Hokkaido do not hold so many leaves on the stem.

Flower forming period: Passed through the stage of the flat apex, it is evident to change the form to a round apex. This represents a stem length of below about 1 to 2 cm. These are the anotomical indication of the transition from leaf forming to flower forming. This transition period is very flexible and extends over the low temperature period. In the late September planting of bulbs imported from Saitama prefecture after digging them up in August, along with slow elongation of the stem, there appeared broadening of the apex at the very beginning of October in Hokkaido. And in the late August planting of bulbs soon after digging them up in Hokkaido, there appeared some broadening of the apex by the end of October along with the very slow elongation. However, there were individual variations in the flower forming of these observations. There appeared evidence of the lack of uniformity both in examination where many bulbs were observed at the same time, and in the appearance of the same periods at the different times. In some of the materials cut off of the stems in August, there appeared the same result on the transition from the leaf forming to the flower forming and the materials left in naturally were delayed more than about a month in flower differentiation. There was almost no growth continued and differentiation of the floral parts during the winter months in Hokkaido.

To find the optimal temperature at this transition period, bulbs, *variety insulare*, 15 to 25 gm., kept at 22° to 25°C for 30 days soon after digging them up in mid August (A), bulbs at 25° to 28°C for 15 days and then at 18° to 22°C for 15 days (B), bulbs at 18° to 22°C for 20 days and then 22° to 25°C for 10 days (C), and bulbs at 10° to 14°C for 20 days and then at 19° to 22°C for 10 days (D) were observed by comparing some differences in the appearance of the differentiation 30 days after planting. In the same manner bulbs kept at 19° to 22°C for 30 days soon after digging them up in mid September (E), bulbs at 25° to 28°C for 15 days and then at 18° to 22°C for 15 days (F), bulbs at 10° to 14°C for 20 days and then at 19° to 22°C for 10 days (G) and bulbs at 7° to 10°C for 20 days and then at 19° to 22°C for 10 days (H) were observed by comparing their development 30 days after planting. Bulbs in B and F resulted in the most advanced stage of flower forming period.

Elongation period: As mentioned above the pre-differentiation stage of flower organs was succeeded by that of differentiation of the first flower and elongation of the stem. This is the reason why most growers presume that the flower bud initial is formed before the stem roots come out and the differentiation of the floral organ occurs after many stem

| Material     | Temperature treatment; (temperature) days  | Stem length 20 days after the treatment (cm.) |
|--------------|--|---|
|              | (22°-25°C) 20 (15°-20°C) 10 ( 5°- 9°C) 20  | 2.0   |
| Dug up on    | $(22^{\circ}-25^{\circ}C)$ 20 $(15^{\circ}-20^{\circ}C)$ 10 $(9^{\circ}-14^{\circ}C)$ 20                             | 4.0   |
| August 15    | $(25^{\circ}-28^{\circ}C)$ 15 $(18^{\circ}-22^{\circ}C)$ 15 $(9^{\circ}-14^{\circ}C)$ 20                             | 5.5   |
| August 15    | $(18^{\circ}-22^{\circ}\text{C}) \ 20 \ (22^{\circ}-25^{\circ}\text{C}) \ 10 \ (13^{\circ}-17^{\circ}\text{C}) \ 20$ | 0.7   |
|              | $(10^{\circ}-14^{\circ}C)$ 20 $(19^{\circ}-22^{\circ}C)$ 10 $(9^{\circ}-14^{\circ}C)$ 20                             | 0.9   |
|              | (19°-22°C) 20 (13°-17°C) 10 ( 6°-9°C) 20   | 6.5   |
| Dug up on    | $(19^{\circ}-22^{\circ}C)$ 20 $(13^{\circ}-17^{\circ}C)$ 10 $(9^{\circ}-14^{\circ}C)$ 20                             | 8.0   |
| September 20 | $(25^{\circ}-28^{\circ}C)$ 15 $(18^{\circ}-22^{\circ}C)$ 15 $(9^{\circ}-14^{\circ}C)$ 20                             | 10.5  |
| September 20 | $(10^{\circ}-14^{\circ}\text{C}) \ 20 \ (19^{\circ}-22^{\circ}\text{C}) \ 10 \ (7^{\circ}-10^{\circ}\text{C}) \ 20$  | 0.3   |
|              | ( 7°-10°C) 20 (19°-22°C) 10 (10°-14°C) 20  | 0.4   |

Table 2. Optimal temperature of the initial stem elongation.

roots arise. Results of some tests to find the optimal temperature of the change to the elongation period was shown in Table 2, using bulbs *variety insulare*, 15 to 25 gm. Although growers misunderstand that a resting period follows the withering of the top, as a matter of fact the inherent resting period does not exist in easter lily bulbs.

Plastic substances in the bulb.

Results of the chemical analyses on protein nitrogen, soluble nitrogen, reducing sugar, non-reducing sugar and starch in the bulb are shown in Figure 6, 7, 8, 9 and 10. Protein nitrogen content reduces until green leaves emerge on the soil and increases by September except reduction at the time of fruiting in August. Protein nitrogen decreases during winter months maybe by consumption for

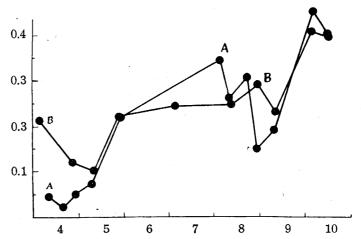


Fig. 6. Fluctuation of protein nitrogen content in the bulbs. A-15 to 20 gm. bulb, B-3 to 5 gm. bulb. Ordinate content, mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

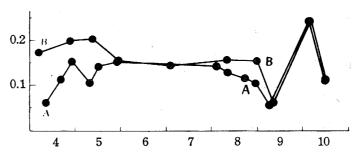


Fig. 7. Fluctuation of soluble nitrogen content in the bulbs. A—15 to 20 gm. bulb, B—3 to 5 gm. bulb. Ordinate content, mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

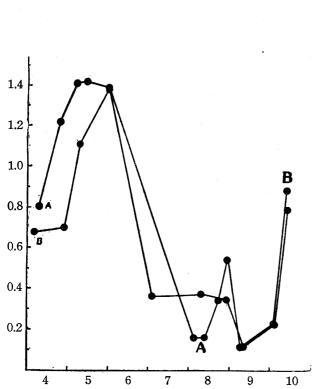


Fig. 8. Fluctuation of reducing sugar content in the bulbs. A—15 to 20 gm. bulb, B—3 to 5 gm. bulb. Ordinate content, mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

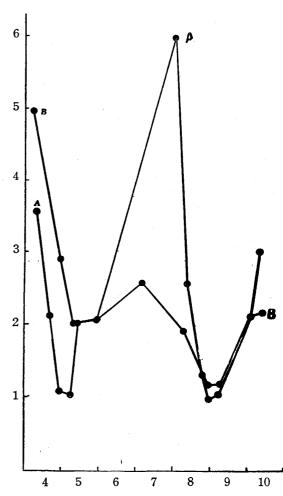


Fig. 9. Fluctuation of non-reducing sugar content in the bulbs. A—15 to 20 gm. bulb, B—3 to 5 gm. bulb. Ordinate content mg. in 0.1 gm. liv ng bulb, abscissa-month (from April to October).

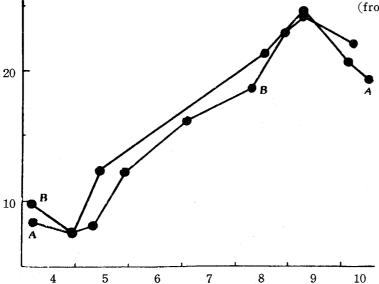


Fig. 10. Fluctuation of starch content in the bulbs. A-15 to 20 gm. bulb, B-3 to 5 gm. bulb. Ordinate-content mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

a source of supply (Fig. 6). Soluble nitrogen content increases, on the contrary, in April and reduces a little in May. It continues constantly in June and July, and reduces again in August or early September. There appears increase again in September and decrease in October (Fig. 7). Comparing with the morphological observations of the bulb development the soluble nitro-

gen content increases twice at the time of new organ formations. Reducing sugar content increases during April and May, namely at the time of new bud and flower formation, at the time of bulb weight reduction. It decreases exceedingly from early June to mid August with the increase of non active substances. Reducing sugar content

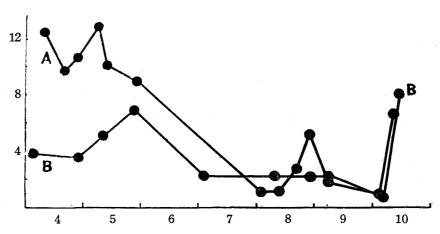


Fig. 11. Periodical change of the C-N ratio by reducing sugar and soluble nitrogen content in the bulbs. A—15 to 20 gm. bulb, B—3 to 5gm. bulb. Ordinate-C-N ratio, abscissa-month (from April to October).

also increases, three times, at the time of flower organ differentiation and the comming low temperature (Fig. 8). On the contrary non-reducing sugar content decreases by mid May. After the growth of tops above soil, it increases exceedingly by the beginning of August and reduces in August with slight increase of reducing sugar. It increases again from the beginning of September (Fig. 9). This may relate to the decrease of starch content in fall (Fig. 10). Starch content increases after the emergence of green leaves and continues fairly constant increase by mid September. It indicates slightly gentle increase before and after flowering. With the witherting there appears decrease of the starch content and it continues during the winter months (Fig. 10). C-N ratio based upon reducing sugar and soluble nitrogen is shown in Figure 11. It is very low during leaf forming period especially from July to September. There appears a tiny peak at the end of August, at the time of fruiting. After stem elongation and floral differentiation it decreases exceedingly while reducing sugar decreases and non-reducing sugar and starch increase on the contrary.

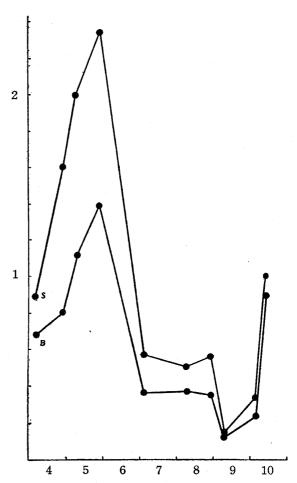


Fig. 12. Fluctuation of reducing sugar content in the bulbs from bulblets (B) and from seeds (S). Ordinate-content mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

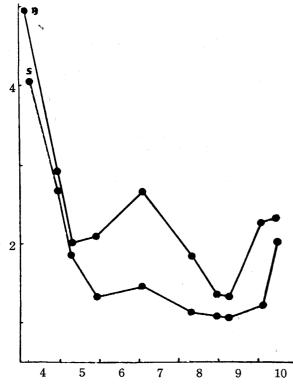


Fig. 13. Fluctuation of non-reducing sugar content in the bulbs from bulblets (B) and from seeds (S). Ordinate content mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

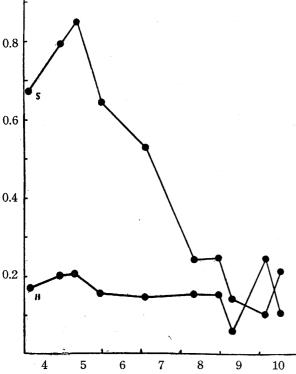


Fig. 14. Fluctuation of soluble nitrogen content in the bulbs from bulblets (B) and from seeds (S). Ordinate-content mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

Contents of reducing sugar, non-reducing sugar, soluble nitrogen and protein nitrogen in the bulb of 3 to 5 gm. raised from seed were compared with those in bulbs from bulblets of 3 to 5 gm. from April to October (Fig. 12, 13, 14 and 15). The bulb raised from seed contained more soluble nitrogen, protein nitrogen and a little more reducing sugar than that from bulblets. Bulbs raised from seed have more reducing sugar than bulbs from bulblets, showing the identical mode of fluctuation (Fig. 12). On the contrary bublets have more non-reducing sugar than bulbs from seed also with the similar fluctuation (Fig. 13). The transition from decrease to increase of the content in bulbs from seed is behind the time of that in bulbs raised from bulblets (Fig. 13). The difference of the soluble nitrogen contents in those two kinds is very conspicuous (Fig. 14) and reduces along with progress of the growth in succeeding year. The fluctuation of soluble nitrogen in bulbs from seed also, are behind the time comparing with those in bulbs from bulblets during September and October. The content of protein nitrogen also shows the lagging of the transition from decrease to increase between September and October (Fig. 15). The protein nitrogen content in bulbs raised from seed in the spring was also surpassed that of bulbs from bulblets.

The C-N ratio based upon reducing sugar and soluble nitrogen contents of bulbs from seed bulblets are shown in Figure 16. The C-N ratio of bulbs raised from seed is always lower than that of the other. The decrease of that of bulbs from bulblets in April does not appear in that of bulbs from seed and the decrease and increase in bulbs

from seed in September and October appears earlier than those of the other.

Contents of these four in double nosed bulbs were compared with those of normal ones, using bulbs after two or three new scale leaves were examined with unaided eyes. The results do not show any constant tendency.

Influence of the external factors.

Searching for the reason of much production of split bulbs in Hokkaido with much snow, temperature and other physical conditions at about new bud forming period, as the mechanical pressure, light, 8 hydrogen ion concentration and humidity etcetera, were examined in the effect to the promotion of split bulb formation.

Mechanical pressure: Materials of about 20 gm. planted in the glass tubes with holed bottom were

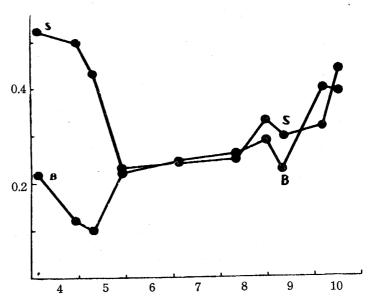


Fig. 15. Fluctuation of protein nitrogen content in the bulbs from bulblets (B) and from seeds (S). Ordinate-content mg. in 0.1 gm. living bulb, abscissa-month (from April to October).

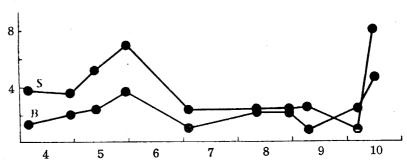


Fig. 16. Fluctuation of C-N ratio by reducing sugar and soluble nitrogen contents in the bulbs from bulblets (B) and from seeds (S). Ordinate-C-N ratio, abscissa-month (from April to October).

specially equipped for giving mechanical pressure to a top of stem of about 3 cm. long from the top of the bulb. About a month later the equipment was removed from the glass tube. The results did not show any difference of split bulb formation between treated and non-treated ones; 33.4% in treated bulbs of 10 to 20 gm., 36.0% in treated bulbs of 30 to 40 gm. resulted in spliting, while 32.2% in non-treated bulbs of 10 to 20 gm. and 33.8% in non-treated bulbs of 30 to 40 gm. on the other hand.

Light: As mentioned above, shallow planting resulted in much production of split bulbs in Hokkaido. If there exists certain difference of split bulb production between exposed (control) and shaded ones at the time of new bud forming, it needs to design further experiments on the light effect. However, the results did not show any particular difference on it. In control, 44% of 6 to 10 gm. bulbs and 80% of 15 to 20 gm. ones produced split bulbs, while 48% of 6 to 10 gm. bulbs and 78% of 15 to 20 gm. ones in shaded bulbs.

Hydrogen ion concentration: Materials of 10 to 15 gm. planted in the glass tubes with the holed bottom were sprinkled with water of pH 4.0 to 8.0 for about a month after the thawing of snow. The percentages of the occurrence of double nosed bulbs by these were; 45% by pH 4.0, 5.0 and 8.0, 40% by pH 6.0 and 7.0, while 45% in control. Laying aside the question of the individual growth, there seems to be no positive relation between the pH and the occurrence of double nosed bulbs.

Humidity: Materials of 10 to 15 gm. planted in the glass tubes with the holed bottom were kept in various soil humidity for 20 days and the occurrence of split bulbs in each of them and in control were compared with each other (Table 3). But if they were kept in the room temperature 15° to 20°C there appeared the quite different results as seen in the Table 4. Judging from these results it might be reasonable to think that the restraining of growth by soil humidity at the time of new bud formation is not able to produce a lot of split bulbs without temperature effect.

| Treatment (percentage of the water capacity) | Occurrence of the split bulb (%) |
|--|----------------------------------|
| (75—80%)                                     | 40.0                             |
| (60-70%)                                     | 40.0                             |
| (60-70%)(45-55%)(80-85%)                     | 49.0                             |
| (75-80%)(30-35%)(75-80%)                     | 53.3                             |
| (75-80%)(55-60%)(75-80%)                     | 40.0                             |
| (75-80%)(45-50%)                             | 46.7                             |
| (75-80%)-(30-35%)                            | 16.7                             |

Table 3. Soil humidity and the occurrence of double nosed bulb (1)\*

<sup>\*</sup> The temperature at soil surface was 0.3° to 23.5°C and in the soil 5 cm. deep, 1.2° to 18.1°C during the experiment.

| Table 4. | Soil | humidity | and | the | occurrence | οf | double | nosed | bulb | (2) |
|----------|------|----------|-----|-----|------------|----|--------|-------|------|-----|
|----------|------|----------|-----|-----|------------|----|--------|-------|------|-----|

| Treatment (percentage of the water capacity) | Occurrence of the split bulb (%) |
|--|----------------------------------|
| (76-80%)                                     | 5.6                              |
| (75-80%)(30-35%)(75-80%)                     | 16.7                             |

Temperature: The plan of experiments on temperature effect is shown in Table 5 with some notes. The results shown in Table 5 indicate that the most exceeding production of double nosed bulbs was in the treatment of low temperature, 1° to 3°C, for 10 days at night only. Treatments of low temperature, 1° to 3°C at each alternate days, 5° to 7°C for 10 days every night only or 1° to 3°C for 5 days, resulted in exceeding production of the double nosed bulbs, while other treatments as low temperature, 1° to 3°C for 1 or 3 days at night only, 5° to 7°C for 1, 3, or 5 days, or 5° to 7°C for 10 days at each alternate day resulted in very low production of double nosed bulbs.

As there appeared a great difference in the occurrence of the double nosed bulbs by different temperature treatments, there existed also a great difference caused by different growth stages on which various treatments were carried out. These differences are repre-

| Weight of bu   |                  |     | 20           | -30 |     | 8–14     |              |     |     | 1.5-4  |        |     |     |     |
|----------------|------------------|-----|--------------|-----|-----|----------|--------------|-----|-----|--------|--------|-----|-----|-----|
| Temperature    | of<br>n, cm.     | 0   | 2            | 4   | 10  | 0        | 2            | 4   | 10  | (Leaf) | (Leaf) | 0   | 1.5 | 3   |
| and treatment* |                  | (a) | (b)          | (c) | (d) | ( e )    | (f)          | (g) | (h) | (i)    | (j)    | (k) | (1) | (m) |
|                | $\mathbf{A}^{-}$ | 8   | 9<br>(4)     | 9   | 4   | 4        | 9<br>(4)     | 8   | 2   | 0      | 2      | 5   | 6   | 2   |
|                | В                | 4   | 3 $(1)$      | 4   | 2   | _        | 3<br>(1)     | 4   |     | 0      | 0      | 1   | 0   | 0   |
| 1-3°C          | С                | 4   | 4<br>(1)     | 7   | 2   |          | 3<br>(1)     | 4   | 2   | 0      | 0      | 1   | 0   | 0   |
|                | D                | 6   | 7<br>(2)     | 8   | 2   | <u> </u> | 6<br>(2)     | 6   | 2   | 0      | 0      | 2   | 2   | 0   |
|                | Е                | 6   | 7<br>(2)     | 9   | 1   | _        | 7<br>(2)     | 8   | 4   | 0      | 0      | 5   | 5   | 2   |
|                | Α                | 6   | 6<br>(3)     | 5   |     | 3        | 6 (3)        | 8   | 2   | 0      | 0      | 3   | 4   | 2   |
|                | В                | 2   | <b>2</b> (0) | 1   |     |          | 2<br>(0)     | 2   | _   | 0      | 0      | 0   | 0   | 0   |
| 5-7°℃          | С                | 2   | 1<br>(0)     | 1   |     |          | <b>2</b> (0) | 2   | 2   | 0      | 0      | 0   | 1   | 0   |
|                | D                | 2   | <b>2</b> (0) | 2   |     |          | <b>2</b> (0) | 2   | 2   | 0      | 0      | 1   | 2   | 0   |
|                | E                | 4   | 2            | 2   |     |          | 3            | 4   | 4   | 0      | 0      | 2   | 3   | 0   |

Table 5. Low temperature treatment plots.

- \* A. Placed in low temperature for 10 days at night only.
  - B. Placed in low temperature for 1 day long.
  - C. Placed in low temperature for 3 days.
  - D. Placed in low temperature for 5 days.
  - E. Placed in low temperature for 10 on alternate days.

Numbers of double nosed bulbs in 10 materials were listed. Parenthesized numbers are of double nosed ones in 10 materials.

sented diagrammatically in Figure 17, 18, 19 and 20. These diagrams show that there appeared the peak at the time of 2 to 4 cm. stem length. When using bulbs, 1.5 to 4 gm., however, there existed the peak at the treatments to those of 0 to 1.5 cm. stem length as in Figure 21.

These results indicate that the A-treatments resulted in the most exceeding split bulb production

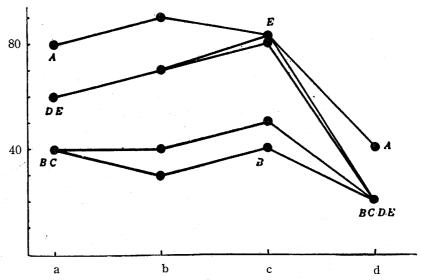


Fig. 17. Occurrence of the double nosed bulb after temperature treatment, 1 to 3°C using bulbs of 20 to 30 gm. cf. Table 5. Ordinate-percentage of the occurrence, abscissa-treatment.

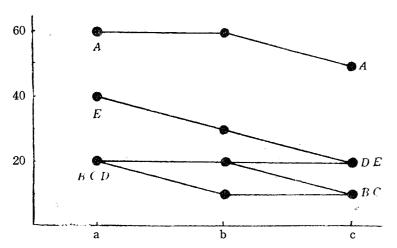


Fig. 18. Occurrence of the double nosed bulb after treatment of 5 to 7°C using 20 to 30 gm. cf. Table 5. Ordinate-percentage of the occurrence, abscissa-treatment.

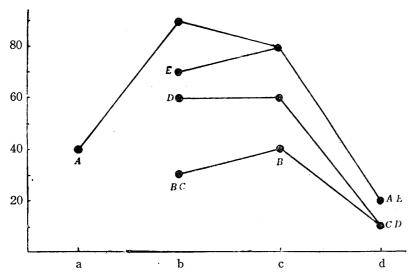


Fig. 19. Occurrence of the double nosed bulb after treatment of 1 to 3°C using bulbs of 8 to 14 gm. cf. Table 5. Ordinate-percentage of the occurrence, abscissa-treatment.

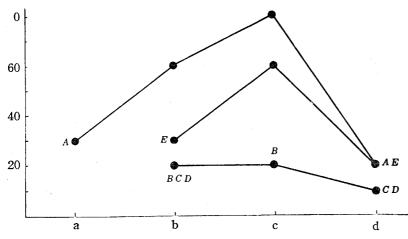


Fig. 20. Occurrence of the double nosed bulb after treatment of 5 to 7°C using 8 to 14 gm. cf. Table 5. Ordinate-percentage of the occurrence, abscissa-treatment.

and the next was the Etreatments. The treatments under the low temperature, 1° to 3°C, were more effective than the others by 5° to 7°C. According to the examination of the results by C- and D-treatments, the effect on split bulb formation increases with addition of the length of time the temperature remains low. In A- and E-treatment, however, there existed the rapidity with which it drops in liue of the difference of the length of time the temperature remains low. The length of time the temperature remains low, therefore, as well as rapidity or fluctuation with which it drops, seemed to be as important as the actual low temperature reached.

Moreover, two different temperature under which materials were kept before treatments,  $2^{\circ}$  to  $7^{\circ}$ C (soil temperature before and after new bud initiation, Saitama) and  $-1^{\circ}$  to  $1^{\circ}$ C (soil temperature under the snow, Sapporo), resulted in different production of split bulbs (Table 5).

Double nosed bulb formation in the propagation by scale leaf:

There ariseo ccasionally two buds close together on one scale leaf and they develop independently. But this is quite different from the so-called double nosed bulb or split bulb. However, there appears sometimes the double nosed bulb formation after the shoot elongation.

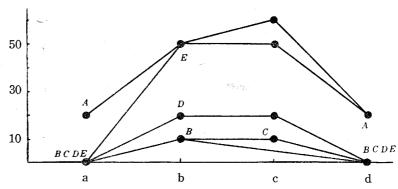


Fig. 21. Occurrence of the double nosed bulb after treatment of 1 to 3°C using 1.5 to 4 gm. cf. Table 5. Ordinate-percentage of the occurrence, abscissa-treatment.

Preparation of materials: Scale leaves were planted in November in usual way, while some of them were soaked in the indoleacetic acid solution of 500 mg./L. for 24 hours just before planting (Table 6). The stem elongation of the new bud on the scale leaves soaked in the solution appeared much earlier than the others. The increase of bulb weight on the scale leaves treated by indoleacetic acid was much more than that of non treated ones, and the increase in the weight of bulbs with early stem elongation was much more than that of bulbs with only rosettes.

| Material                                       |     | 105 days<br>after<br>planting | 125 days | 140 days | 165 days | 200 days | 300 days |
|--|-----|-------------------------------|----------|----------|----------|----------|----------|
| 2-year scale leaf                              | 268 | 0                             | 0        | 0        | 14       | 16       | 16       |
| 1-year scale leaf                              | 84  | 0                             | 0        | 1        | 3        | 4        | 5        |
| 1-year scale leaf                              | 109 | 1                             | 3        | 6        | 11       | 11       | 11       |
| 1-year scale leaf                              | 120 | 4                             | 4        | 5        | 13       | 15       | 13       |
| 1-year scale leaf                              | 135 | 4                             | 5        | 12       | 14       | 17       | 18       |
| 1-year scale leaf                              | 160 | 4                             | . 5      | 5        | 10       | 13       | 13       |
| 2-year scale leaf treated by indoleacetic acid | 29  | 5                             | 6        | 10       | 13       | 16       | 20       |
| 1-year scale leaf treated by indoleacetic acid | 121 | 26                            | 39       | 58       | 86       | 94       | 95       |

Table 6. Days up to the stem elongation in scale leaf propagation.

Double nosed bulb formation: It is only when the stem elongated by April that there appeared the split bulb formation in the case of propagation by scale leaf. The low temperature treatments were applied to the materials which had elongated stem of about 3 cm. from the base to the top of the stem. The results shown in Table 7 intimate that there may be a certain relationship between temperature at that time and accidental new bud formation as former cases.

Plastic substances in scale leaves: Generally speaking, the inner scale leaves contain active nitrogen in great quantity, while the outer ones are rich in active sugar as seen in Table 8. On the contrary the outer ones contain protein nitrogen in great quantity, while the outer ones are rich non-active sugar, too. Speaking of the distribution of these substances in each scale leaf, the active substances, soluble nitrogen and reducing sugar,

| Temperature                    |   |   | 0-2°C |   |   |   |   | No |   |     |           |
|--------------------------------|---|---|-------|---|---|---|---|----|---|-----|-----------|
| Treatment                      | A | В | С     | D | E | A | В | С  | D | Е   | treatment |
| Number of material             | 5 | 5 | 5     | 5 | 5 | 5 | 5 | 5  | 5 | 5   | 25        |
| Number of double<br>nosed bulb | 3 | 1 | 1     | 2 | 3 | 1 | 0 | 0  | 0 | , 1 | 0         |

Table 7. Temperature effect on the occurrence of the double nosed bulb in the scale leaf propagation\*

Table 8. Substances in the scale leaves at planting (mg. in 0.1 gm. fresh scales).

| Substances         | Upper part of<br>the outer<br>1-year scale<br>leaves | Basal part of<br>the outer<br>1-year scale<br>leaves | 2–year scale<br>leaves | Outer 1-year scale leaves | Inner 1-year scale leaves |
|--------------------|--|--|------------------------|---------------------------|---------------------------|
| Soluble nitrogen   | 0.0001   | 0.1233   | 0.0052                 | 0.0970                    | 0.1890                    |
| Protein nitrogen   | 0.4786   | 0.3985   | 0.5761                 | 0.0594                    | 0.4528                    |
| Reducing sugar     | 0.0430   | 0.2196   | 0.4168                 | 0.0594                    | 0.0577                    |
| Non-reducing sugar | 0.8011   | 1.6326   | 1.0089                 | 1.3308                    | 0.9852                    |
| Starch             | 20.0165  | 25.5758  | 22.0111                | 23.3567                   | 14.0101                   |

are rich in its basal part. Only protein nitrogen is richer in the upper part than in the basal part. Non-reducing sugar also is rich in the basal part, while the starch contents in both of these are not so different.

To examine the difference of the contents of these substances in the bulbs, normal and double nosed ones, the analyses were done at the end of August (Table 9). According to the results, bulbs with rosettes only were rich in soluble nitrogen and not in others. Bulbs without spliting were very rich in reducing sugar, non-reducing sugar and strach,

Table 9. Substances in the new bulbs produced by scale leaf propagation (mg. in 0.1 gm. new fresh bulb).

| Material                              | Soluble<br>nitrogen | Protein<br>nitrogen | Reducing<br>sugar | Non-reducing<br>sugar | Starch  |
|---------------------------------------|---------------------|---------------------|-------------------|-----------------------|---------|
| Normal ones with elong-<br>ated stem  | 0.1750              | 0.3076              | 0.9881            | 4.3020                | 23.1125 |
| Double nosed ones with elongated stem | 0.2155              | 0.3098              | 0.2935            | 1.7298                | 20.5561 |
| Bulbs rosettes only                   | 0.2665              | 0.2279              | 0.2244            | 1.7321                | 17.9984 |

while not in soluble nitrogen. Bulbs with double nosed daughter bulbs were richer in soluble nitrogen than the normal bulbs and almost similar in protein nitrogen, while very low in the others. Judging from these results it seems necessary to let them get into the stem elongation as soon as possible, by spring in Hokkaido.

### 4. Discussion

It is necessary to know the periodical development of the easter lily bulb before a discussion on the double nosed bulb formation. The development of the bulb of 3 inches circumference planted in fall was diagramatized in Figure 22 with some notes on the

<sup>\*</sup> cf. Table 5.

growth periods in Hok-kaido. The easter lily bulb in the fall consists of two parts, one-year-tissues, bulb axis and scale leaves (B) and two-year-tissues, bulb axis and scale leaves (A) as mentioned above. The terminal bud of the



Fig. 22. Behavior of the easter lily bulb. A, B, C and D show the bulb generations. a—embryonic period, b—scale leaf forming, c—foliage leaf forming, d—flower bud forming, e—extension, f—aquired rest, g—extension and flower forming again, h—outer exfoliation and inner enlargement, i—flowering, j—fruiting, k—exfoliation period, l—complete exfoliation.

B changes from leaf bud to flower bud caused by low temperature, the stem grow continually little by little and in next April there arises a new bud (C) on the axil of the last of the scale leaves of the B. And so, the bulb now, consists of three parts, A, B and C. The A is in the exfoliation period, offering their reserve substances to the leading growth of the whole bulb, while the growth of C is the very preliminary. As temperature rises the B enters into the period of elongation, flowering and fruiting by August or September. The exfoliation period of the A continues until about mid June. The outer scales of B also may offer their reserve substances to the flowering and fruiting after exfoliation of the C, while inner ones are enlarged during summer. Thus scale leaves and bulb axis become exfoliated from the outer part. After one year from planting all of the A and 2/5 to 2/3 of the B tissues, scales and axis all together, are decayed in this way. It is great importance to confine the exfoliation of outer scales of the B to the minimum. After fruiting of B, the terminal bud of the C enters into the foliage leaf forming stage from the scale leaf forming. The transition from leaf forming to flower forming is carried forward upon the opportunities of the change of internal conditions and of the comming of low temperature. The time of this transition is different from plans of digging up and planting and the interval condition between those when comparing with that in the same location of northern climates, while different from location when the same cultivation way is employed. Because of the low temperature after the transition or the pre-differentiation of the flower bud, this transition period continues flexible and the very slow extension of stem is carried forward simultaneously during the cold season. After digging it up the B tissues are in the exfoliation period and extinguished by next June. Thus the diagram indicates that the same age tissues of the bulb continues over three calendar years and never continue more than that.

It can be said that the new bud formation in the axil of the innermost layer of the scales on the bulb axis is one of atrophied collateral bud which has regained its vital functions, partly and contingently. If usual lateral bud, it is unwarrantable to a great lack of uniformity on the location of new bud in the axil and if adventitious bud, it is unwarrantable to the constant appearance in the axil only. Anatomically, there arises generally one axilliary bud in the axil of the last of scale leaves on the bulb axis. As mentioned above, speaking of larger bulb, there exists almost equal possibility of new

bud formation in the last three axils which hold the room of new bud formation in the axil of elongated axis. Usually only one arises, while the others remain flat.

The accidental occurrence of double nosed bulbs is not a clonal character, but physiological one caused by the development of an extra bud in the axil of the other, or at times, of the same scale leaf, growing beside the first one, forming together the double nosed bulb afterwards. Splitting of flower buds is caused by aphid injury (Post, 1941). Drying, excess water, excess fertilizer and a sudden drop in temperature have not caused splitting and other causes have not yet been discovered. Splitting of the bulb, however, is not caused by aphid injury or any other handicap after stem roots arise.

The new bud which arise in April in Hokkaido, from December to February in Saitama prefecture, has six periods, embryonic period, leaf forming period, flower forming period, elongation period, flowering and fruiting period and exfoliation period, from the beginning of the new bud to the complete exfoliation in June of the year after next. These periods do not exist independently and also there exists the transition on which two periods overlap as in flower forming and elongation period (Fig. 22). Results obtained by Blaauw and co-workers on development and temperature summarized the most important phenomenon, thermoperiodicity. of greater significance for the bulb industry was the fact that some of the most important stages in the development of a bulb were passed during its storage period. Adjustments to the treatment in storage are possible, which improve the later performance of the bulb in the field. Judging from some secondary observations on development and temperature in this studies of double nosed bulb formation, the greater significance for the bulb production is the fact that some of most important stages of the development were passed in the soil during winter months, after planted in fall. The stem elongation during winter and early spring in Saitama prefecture shown in Table 10 indicates that there exists slow elongation under the soil temperature 2° to 8°C in February. The new bud formation of larger bulbs in Saitama prefecture appears during winter months, December to February, and in April in Hokkaido. The soil temperature in about 5 cm. deep, at this new bud formation period, is  $1^{\circ}$  to  $5^{\circ}$ C in Saitama, and 0° to 24°C in Hokkaido. The fluctuation of temperature in Saitama is rather moderate, while that in Hokkaido is keen from a physiological point of view. A sudden change of temperature between morning and evening characterizes the latter as well as the minimum temperature. There appeared about 12 days in April in Hokkaido, on which the fluctuation of temperature of each day is above 10°C in variation and marks below

Table 10. Stem growth in Saitama prefecture in winter (cm.)

|         | Bulblets |     |      | Bulbs of 3-4 inches circumference |     |      | Bulbs of 5-6 inches circumference |      |      |
|---------|----------|-----|------|-----------------------------------|-----|------|-----------------------------------|------|------|
| Date    | Mx.      | Mn. | Ave. | Mx.                               | Mn. | Ave. | Mx.                               | Mn.  | Ave. |
| Jan. 25 | 0        | 0   | 0    | 5.5                               | 2.0 | 3.1  | 6.0                               | 2.0  | 4.0  |
| Feb. 29 | 0.8      | 0   | 0.1  | 8.3                               | 2.1 | 4.4  | 8.5                               | 2.2  | 4.6  |
| Mar. 25 | 5.3      | 0   | 3.7  | 11.0                              | 5.0 | 7.8  | 13.5                              | 9.0  | 10.4 |
| Apr. 23 | 13.5     | 7.3 | 11.4 | 18.0                              | 8.0 | 15.6 | 24.5                              | 18.0 | 20.2 |

7°C as minimum. Judging from the soil temperature in Hokkaido and the experimental results on the relation of temperature to the new bud formation, it is reasonable to consider that the low soil temperature and its fluctuation give rise to the accidental formation of additional new bud. It can be said that low temperature and the trimming of roots tend to induce the double nosed bulbs, which coincides nearly with time of the new bud formation in the mother bulb. Other physical conditions such as the light, humidity, hydrogen ion concentration etcetera, if acting singularly, are unable to induce the double nosed bulbs. Thus these two external factors treated timely resulted in much production of the double nosed bulbs. It can be said that they brought about certain condition of active substances available for new bud formation. One of them may be the special substance.

Growers have afraid that the double nosed bulb is not desirable for forcing and marketing. According to my studies, however, this grower's concern is surely groundless. Occurrence of the double nosed bulb and meagre development of the bulb do not always correlated. The very frequency of inadequate bulb thickening is a serious drawback of easter lily bulb industry in Hokkaido. Therefore, it is important in the easter lily bulb industry in northern climates to make it possible to get enough enlargement of the bulb by improving the grower's practices. Soil temperature of 5 cm. deep in Saitama, that in

Hokkaido, optimal soil temperature obtained in the experiments and desirable soil temperature in Hokkaido were diagrammatized in Figure 23. The soil temperature in Saitama is very close to the optimum except that in winter. But it is possible to keep it higher by mulching etcetera and is recommended in bulb production in Saitama to plant

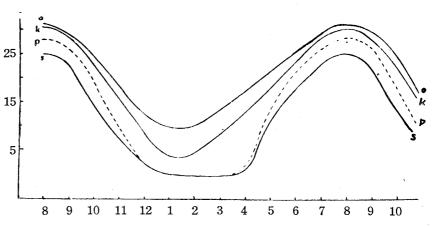


Fig. 23. Soil tempertaure in Easter lily bulb industry. s—soil temperature of 5 cm. deep, Sapporo. k—in Saitama. o—optimal soil temperature of 5 cm. deep. p—available soil temperature of 5 cm. deep in Sapporo. Ordinate-temperature (C), abscissa-month (from August to next October).

the bulb in early October without any particular temperature treatment because of the soil temperature succeeded available for starting stem elongation. The soil temperature in Hokkaido, however, is so low even in summer months that it is necessary to try to keep it higher from very early spring to late fall. Furthermore, there exists a discrepancy between the periodical development of the bulb and the seasonal fluctuation of soil temperature, which is particularly tremendous in early spring and late fall in Hokkaido. To keep the soil temperature higher is surely favourable to the bulb development and also effectual to getting soil temperature closer to the optimum.

The relation between the periodic bulb development and the metabolic change of substances in the bulb was studied by chemical analyses, together with morphological observations simultaneously. The analyses were made on the following fractions, protein nitrogen, soluble nitrogen, reducing sugar, non-reducing sugar and starch, during the period from the beginning of April to the mid October. Generally speaking, the inner part of the bulb contains available nitrogen in great quantity, while the outer part of the bulb is rich in available sugars. As to each scale leaf, the active reserve substances, soluble nitrogen and reducing sugar are rich in its basal part. Total nitrogen content increases gradually from early spring to August, with a slight drop at the fruiting stage; then it increases again, until the recede in October. At the time of rapid growth, from April to June, reducing sugar predominates exceedingly. Large amount of non-reducing sugar is recognized in summer and winter. Starch increases from spring onwards, but decreases after early September. If the reserve substances of young plants from seed and from bulblets are compared, the bulb of the former contains more soluble nitrogen, protein nitrogen and a little more reducing sugar than the latter, while the former contains more non-reducing sugar than the latter. But these differences disappear in the progress of growth. In comparing the reserve substances of normal bulbs with those of double nosed ones, the difference in these contents do not show any legible tendency.

From what has been said it is seen that it seems reasonable, therefore, to plant bulbs in late August or early September in Hokkaido, though the condition is somewhat complicated. As for the differentiation of root and bud, it is suitable in Hokkaido to dig up the bulb while the shoot is still green and plant the bulb in the fields. When the formation of the foliage leaves in the bulb is manifested and the soil temperature tends to be 15° to 23°C, the bulbs should be planted quickly, different from the warmer regions, where no such treatment is needed. In short, to reduce the production of double nosed bulbs and to produce better bulbs, the time of planting should be determined in accordance to the soil temperature and the phase of the development.

#### 5. Résumé

- 1. In the northern climates of Japan, as in Hokkaido, the bulb production of *Lilium longiflorum* suffers from the following two defects, namely, reduction of size and production of so-called double nosed bulbs. To elucidate the condition of the formation of the double nosed bulbs, the various phases of the development of normal and diverse bulbs were studied from the morphological and physiological point of view, using two varieties, *var. takesima* and *var. insulare* as materials.
- 2. Anatomically, there appear generally two or three axils with equal chance for new bud initations, but usually only one develops, while the others remain flat. The accidental occurrance of double nosed bulbs is not a clonal character, but a physiological one caused by the development of an extra bud in the axil of the same or other scale leaf growing beside the first one, forming together the double nosed bulb afterwards.
  - 3. The life history of the individual bud from the beginning to the end may be

divided into the following six periods, namely, embryonic period, leaf forming period (scale leaf forming period and foliage leaf forming period), flower forming period (the initiation period and differentiation period of the flower organs), elongation period, flowering and fruiting period, and exfoliation period.

- 4. In Hokkaido the commencement of the embryonic period is recognized mostly in April when the mother bud attains about 2 cm. in height, forming the primordium in the axil of the innermost scale leaf on the bulb, while in Saitama prefecture it occurs December to February. After the formation of foliage leaves (optimal temperature 24° to 30°C) in late summer in Hokkaido and in Saitama prefecture, the initiation of the embryonic flower bud (opt. temp. 18° to 23° C) occurs in October or November in Hokkaido (in Saitama, November).
- 5. The time of differentiation varies, however, according to the time of digging up and planting, though the slow elongation of the stem goes on invariably. This elongation of the stem with temperature optimum of 9° to 14°C at the very beginning is retarded by the low temperature of winter in Hokkaido, while in Saitama it goes on incessantly. According to my observation, the inherent resting period of easter lily bulbs does not exist.
- 6. The relation between the periodic bulb development and the metabolic change of substances in bulbs was studied by chemical analyses, together with morphological observations simultaneously. The analyses were done on the five fractions, protein-N, soluble-N, reducing sugar, non-reducing sugar and starch, during the period from the beginning of April to mid October.
- 7. Generally speaking, the inner part of the bulb contains active nitrogen in great quantity, while the outer part of the bulb is rich in active sugar. As to each scale leaf, the active reserve substances, soluble nitrogen and reducing sugar are rich in its basal part. Total nitrogen content increases gradually from early spring to August, with a slight drop at the fruiting stage; then it increases again, until the final decline in October. At the time of rapid growth, from April to June, reducing sugar predominates exceedingly. Large amount of non-reducing sugar is recognized in summer and winter. Starch increases from spring onwards, but decreases after the early September.
- 8. If the reserve substences of young plant from seed and from bulblet are compared, the bulb of the former has more soluble nitrogen, protein nitrogen a little more reducing sugar than the latter, while the former has more non-reducing sugar than the latter. But these differences disappear in the progress of growth.
- 9. In comparing the reserve substances of normal and double nosed bulbs, the difference in these contents does not show any regular tendency.
- 10. Low temperature and severance of roots tend to induce the double nosed bulbs, which coincides nearly with that of the new bud formation. Other physical conditions, as mechanical pressure, light, humidity, hydrogen ion concentration etcetera, if acting singularly, are unable to induce the double nosed bulbs.
  - 11. Most of double nosed bulbs in Hokkaido may be derived from the following two

factors; (1) the low temperature of soil of 5 to 10 cm. deep and (2) its rapid drop from late fall to early spring.

- 12. The degree and the duration of low temperature treatment are both responsible for the occurrence of double nosed bulbs, the 1° to 3°C treatment being more effective than 5° to 7°C treatment a greater drop being more effective than a less one.
- 13. Accordingly, shallow planting is liable to form a large number of unexpected double nosed bulbs. In the northern climates of Japan, it is therefore unavoidable to meet with the double nosed bulbs.
- 14. It seems reasonable, therefore, to plant bulbs in late August or early September in Hokkaido, though the condition is somewhat complicated. As for the desirable differentiation of root and bud, it is suitable to dig out the bulbs while the shoot is still green and plant the bulbs in the fields. When the formation of the foliage leaves in the bulb is manifested and the soil temperature tends to be 15° to 23°C, the bulbs should be planted quickly, different from the warmer regions, where no such treatment is needed.
- 15. To reduce the production of double nosed bulbs, the time of planting should be determined in accordance with the temperature and the phase of the development. The double nosed bulb is not so serious for forcing and bulb industry, when grown enough. There may be special use with them. It seems more recommendable to make efforts to produce substancial bulbs than to try to get out from the production of double nosed bulb.

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