

**ANATOMICAL AND BIOCHEMICAL CHANGES IN
NARCISSUS BULBS DURING SUMMER
STORAGE AT VARIOUS TEMPERATURES**

By
D. ^{and} VICTOR LUMSDEN

**Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for
the degree of Doctor of Philosophy**

1935

UMI Number: DP70065

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI DP70065

Published by ProQuest LLC (2015). Copyright in the Dissertation held by the Author.

Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

TABLE OF CONTENTS

	Page
Introduction.....	1
Review of Literature.....	6
Material and Method.....	9
Results.....	20
Discussion.....	36
Summary.....	45
Figures and Explanations	
of Figures.....	48
Literature Cited.....	76
Acknowledgments.....	83

INTRODUCTION

Narcissus bulbs continue their development and growth during the summer; the investigations reported herein show that bulbs which are out of the ground during the summer, either in storage or in transit, can be significantly affected by storage temperature. Bulbs which are to be planted in the fall for unaccelerated spring flowering do not require special storage conditions if they can be kept in a dry and fairly cool location with good aeration. The treatment of bulbs which are to be forced into early bloom, however, presents a different problem.

Commercial bulb growers and others handling bulbs are aware of the effects of temperature and take advantage of this in order that flowers from these bulbs may be forced into bloom and be available sufficiently early to reach a more favorable market. It is also known that bulbs may be injured so that they will fail to blossom the following spring if they are subjected to an unfavorable temperature for even a short time while they are in

2.

storage during the summer. In the past when narcissus bulbs were shipped from Europe in large quantities, heating of the bulbs in the holds of ships had to be avoided or a poor flowering response of the bulbs would result.

Because all bulbs entering into commerce in any year require a period of storage from the time they are harvested until they are again planted out, it is of interest to mention the number of narcissus bulbs annually handled by the trade in the United States. While there is at present no agency from which complete and accurate statistics can be obtained concerning the number of narcissus bulbs now being stored and transported in the United States, nevertheless it is possible to refer to statistics which will give some idea of the importance of this industry.

Prior to the ban which was placed on the importation of narcissus from abroad on January 1, 1926, a record of the number of narcissus bulbs imported into the United States was a fairly good index of the magnitude of the number of bulbs handled and planted in any given year.

3.

Statistics published in 1928-30 (27) show that the importation of narcissus bulbs into the United States during 1922 and 1923 averaged approximately 77,000,000 bulbs per year. Figures for 1924 and 1925 average about 25% higher. This sudden gain, however, can be attributed to the fact that the announcement was made in 1924 that wholesale importation of narcissus bulbs would be prohibited starting in 1926. The bulb interests were attempting to build up large stocks of bulbs for establishing a bulb industry within the United States.

Since 1926 there have been no means of determining the number of narcissus bulbs moving in commerce each year. A report in 1930 (28), however, showed that there were more than 273,000,000 narcissus bulbs growing commercially in the United States in 1929. Growers' estimates indicated that 20 to 30 per cent of this number were available for shipment.

4.

A survey of the bulb industry in the United States issued in March 1931 (20) showed that there were planted for harvest in 1930, 165,000,000 bulbs, and the growers' estimate of bulbs for sale from the 1930 harvest was about 39,000,000 bulbs. This estimate, however, is acknowledged to be far below the actual quantity due to the small number of growers who replied to the questionnaires sent out.

It has recently been announced by the U. S. Department of Agriculture that the present ban on the importation of narcissus bulbs will be lifted on December 15, 1936. Just what effect this will have on the production of these bulbs within the United States is problematical. Regardless of where these bulbs are grown--whether in the United States or abroad--the task of storing the bulbs until they are again planted still remains.

The purpose of gaining information relative to the anatomical and biochemical changes which occur in bulbs as a result of storage at different temperatures prompted the work described below. This investigation

5.

presents data showing basic changes which occur in narcissus bulbs which at least contribute to acceleration or damage of the flowering bulbs as previously mentioned. It reports anatomical changes and carbohydrate transformations which occur under conditions of controlled temperature during the time the so-called dormant bulbs are normally subjected to forcing treatment.

It is hoped that this investigation will be a contribution to the knowledge of the behavior of bulbs during storage and also to the determination of the most satisfactory conditions under which bulbs may be stored to promote satisfactory flowering at a desirable time.

REVIEW OF LITERATURE

The literature covering the genus *Narcissus* is extensive. A comprehensive bibliography has recently appeared in *The American Daffodil Year Book* (1), in which is given a list of the outstanding papers on this plant.

Detailed investigations covering experimental storage treatments, with or without the aim of promoting early flowering, are not frequently found in the literature. One of the outstanding workers in this field was Dr. David Griffiths. In 1930 he discussed (7) the subject of forcing bulbs and stated that the rate of development of the bud within the bulb depends upon the storage temperature. Within the last few years additional articles have appeared in florists' and seedsmen's trade papers (6), (8-17), in which observations have been recorded concerning the behavior of bulbs, particularly *narcissus*, subjected to various conditions during their storage period.

7.

Hasselbring (19) in 1932 pointed out that the hot-water treatment of narcissus bulbs for the control of eelworms and other parasites resulted in various physical and chemical changes within the bulbs. From analyses of bulbs made at intervals during the storage period he concluded that hot-water treatment at the middle of the storage season also resulted in accelerated activity in the bulbs so treated. Van Slogteren in Holland (30) expressed the opinion that the hot-water treatment may have a stimulating effect on the development of narcissus bulbs during storage.

The writer in 1932 reported (23) that significant changes occurred in the development of the flower bud within the bulb during the storage period. In 1932 Blaauw et al. in Holland (3) also reported on the effect of temperatures on the elongation of the scapes and development of the flowers of narcissus bulbs. In the same year in Holland Beijer and van Slogteren (2) published on the forcing of narcissus bulbs and pointed out what the optimal storage temperature conditions were for the varieties with which they were working.

8.

In 1933 Huisman and Hartsema in Holland (22) issued a publication in which the complete development of the narcissus bulb was portrayed throughout the year. Also in 1933 van Slogteren (31) reported on experiments with the forcing of narcissus. He also showed that temperature played an important part in the forcing of narcissus bulbs.

Investigations of the carbohydrate changes in other flowering bulbs were reported in Pinkhof's publication of 1930 (26), which covered biochemical changes occurring in tulips. This study concerned the so-called wilting disease of tulips.

From the existing literature it is well established that narcissus bulbs can be affected materially by the temperature at which they are maintained during summer storage. It remains to determine in a more detailed way the anatomical and biochemical changes that occur in bulbs stored at different temperatures and any correlations which may exist between these and the responses secured during forcing.

MATERIAL AND METHOD

The bulbs used in this investigation were the self-colored trumpet variety, King Alfred, Narcissus pseudonarcissus L. They had been propagated at the Bulb Culture Station, Bellingham, Washington. During the summers of 1930 and 1932 bulbs were shipped to Arlington Experiment Farm, Rosslyn, Virginia, and kept until planting time in the bulb storage house. The two shipments were planted in the field the fall of these years. In each case the bulbs were dug the following spring at the proper time and prepared for experimental storage. This report covers anatomical and biochemical changes as well as ultimate forcing responses in the bulbs harvested in 1931 and the others harvested in 1933. Samples were not taken for analysis in 1932.

In 1931 the bulbs were harvested on June 30, dried as described later, and on July 21 placed in storage at 80° F. Samples for biochemical and anatomical studies were taken from these bulbs at two-week intervals during the storage period. On September 2 one half of the bulbs remaining were placed at 50° F., while the balance were retained in the 80° F. room. During this second period samples for study were also taken as before.

10.

In 1933 the same general procedure was followed, with some variation in dates due to the fact that the bulbs matured earlier in the field. The bulbs were dug on June 8. But they required longer to dry and were not put into storage until July 21, the same date of the month as the 1931 bulbs. The storage temperature was again held at 80° F. Samples were taken as before, but at longer intervals. On September 5 three-fourths of the bulbs remaining were removed from the 80° F. temperature. These were divided into three equal lots and placed under controlled storage temperatures of 59°, 50°, and 40°F. Again sampling was done, the final samples being taken from the bulbs on October 31.

Storage Conditions and Sampling Procedure in 1931. *

In 1931, as already mentioned, the bulbs were harvested on June 30. The partly spent foliage was removed at this time and the bulbs placed one layer deep on wire mesh-bottomed trays to dry in the bulb storage house. These trays were elevated from the

* The late Dr. Heinrich Hasselbring assisted in the sampling and in the sugar analyses of 1931.

11.

floor to permit air circulation from beneath. On July 17 the bulbs were sufficiently dry so that the roots and outermost scales could be removed with a slight rubbing. On July 18 the bulbs were washed and again spread out on the trays to permit rapid drying. An oscillating fan was used to hasten the evaporation of this surface moisture.

On July 20 the bulbs for subsequent analysis were sorted. 34 comparable samples of 40 bulbs each, representing the entire range in bulb size, were selected, 32 for biochemical studies and 2 for forcing. The average weight of the bulbs in 1931 was 100.2 gms. Eleven samples of 10 bulbs each of the median size were chosen for anatomical study. These samples were each placed in mesh bags to facilitate handling.

The next day, July 21, the bags of bulbs were placed in an 80° F. storage temperature. During the entire period of storage at 80° F. the relative humidity gradually dropped from 70% to 52% with the seasonal change in weather. There was practically no fluctuation in the relative humidity from week to week, the curve showing a steady decline.

12.

On July 22 the bags of bulbs had come to equilibrium with room temperature and each of the 32 bags intended for ultimate analysis was weighed. Every two weeks throughout the storage period the bags of bulbs were weighed to determine shrinkage. On July 23 four bags, quadruplicate samples, were taken to the laboratory and analyzed separately. Every two weeks thereafter four of the remaining bags were used for analysis.

On September 2 one-half the bags left in storage were transferred from the storage room at 80° F. to a storage room at 50° F. The relative humidity in this room fluctuated between 70 and 74 per cent. Of the four bags taken for analysis subsequent to September 2, two bags of bulbs were taken from the higher storage temperature and two from the lower.

While the external appearance of the bulbs in storage at 80° F. throughout the storage season did not change, those removed to 50° F. on September 2 showed unmistakable signs of development externally as the season progressed. On October 1 they showed a thickened callus around the basal plate within which the beginning of roots could be seen on cutting. On October 18 the roots were observed to have pushed through the callus, mostly on one

13.

side, while on October 29, the last day of storage, the roots were projecting out about 1 cm. and forming a dense crown.

Of the 34 bags of 40 bulbs each originally sorted out, 32 were used for biochemical analysis. One of the remaining two was kept at 80° F. throughout the storage period and the other removed to the 50° F. room on September 2. On October 30 these two bags of bulbs were forced to determine their flowering responses after treatment.

1931 Analytical Procedure

In the preparation of the bulbs for chemical analyses, the dry scales, tips, and dry parts of the basal plates were removed. The bulbs were quartered and ground twice through a meat chopper, which forced the pulp through a perforated plate in which the holes were 3 mm. in diameter. As the sticky mass coming from the chopper could not be mixed or quartered, it was reground in order to insure a homogeneous mixture. From the tissue thus mixed, samples for analysis were weighed out. For moisture determinations of the mixture samples of approximately 10 gms. were accurately weighed in weighing bottles and then covered with absolute alcohol. Later this alcohol was evaporated off the samples at 60° C.; they were then dried in a vacuum oven at 70° C. and 16 mm. pressure of mercury in a slow current of dried air. The drying was continued until the weight of the remaining tissue reached an equilibrium and fluctuated within narrow limits depending upon the relative humidity at the time the weighings were made.

Twenty-five gram samples for sugar determinations were washed into beakers with 100 ml. of 95% alcohol. Five-tenths of a gram of calcium carbonate was added to each sample. The beakers were heated to boiling for a few minutes and allowed to stand for 10 days, whereupon the alcohol was

decanted into 250 ml. volumetric flasks. The residues were covered with absolute alcohol and allowed to stand overnight in a desiccator. The material thus hardened was ground in a mortar and extracted once by boiling with 70% alcohol for 30 minutes, and for two periods of eight hours each in soxhlet extractors. This procedure was adopted following earlier findings of Hasselbring (19) that interfering substances in the bulbs made the quantitative separation of the sugars difficult.

The carbohydrate determinations were carried out as described by Hasselbring in a former paper (18).

To determine the amount of acid-hydrolyzable substances in the bulb tissue, ten gram samples were covered with 100 ml. of absolute alcohol and stored in flasks whose stoppers were sealed with paraffin. Ultimately the extracts from the samples were decanted through porcelain extraction thimbles and the residues then transferred to and ground in a mortar under absolute alcohol. The ground material was then returned to the thimble. The material was further extracted with 500 ml. of cold 70% alcohol added in 10 ml. portions to complete the removal of soluble carbohydrates. Acid-hydrolyzable substances in the residues were determined by the acid hydrolysis method of Sachsse (29). All the data on carbohydrates were calculated in terms of grams of hexoses in 100 grams of bulb tissue at the time of preserving the tissue for analysis.

1931 Anatomical Methods

From the bulbs mentioned earlier as being reserved for anatomical studies, lots of ten were taken at approximately bi-weekly intervals during the storage season to study anatomical changes within the bulbs during the period of storage and at the temperatures as stated.

When the bulbs were taken from storage the majority of the scales were stripped from the bulbs as described in an earlier paper (23), so that at most only two or three of the innermost scales were left enveloping the developing flower. These pieces were submerged in formalin acetic alcohol (5 parts glacial acetic acid, 5 parts commercial formalin, 90 parts of 70% alcohol) as recommended by Chamberlain (4) for killing and preserving until the material could be embedded. Samples were taken for these studies on the following dates: July 1, 15; August 1, 17, 24, 31; September 8, 16, 24; October 2, 12.

1933 Sampling Procedure

In 1933, as mentioned before, the bulbs were harvested on June 3, the same procedure for harvesting and drying being employed as for the 1931 bulbs. This year it was again on July 17 that the bulbs were dry enough to clean despite the earlier harvesting date.

In the washing of the bulbs in 1933 an added precaution was taken to attempt to control basal rot which was noted when the bulbs were sampled in 1931. To this end the bulbs for the work were divided into 2 lots after they had been washed in water; each of these 2 lots was separately immersed for one hour in a barrel containing 1 oz. mercuric chloride to 25 gallons of water. The bulbs were then again spread one layer deep on wire mesh-bottomed trays and the surfaces of the bulbs thoroughly dried.

After being dried the bulbs were divided into representative samples as described for the 1931 work. Twenty-two bags of 40 bulbs each were prepared for the biochemical analyses. Four bags of 20 bulbs each were provided for forcing, and 11 bags of 5 bulbs each were provided for anatomical study during the storage period. The average weight of each bulb for 1933 was 112.7 gms.

On July 21 the bulbs were placed in a constant temperature room at 80° F. and on July 24 all samples for the biochemical studies were weighed. On this date also five bulbs were prepared for anatomical study.

1933 Analytical Procedure

On July 25 the first two bags of 40 bulbs each were prepared for analysis as described in the 1931 work with the following changes: Two 50 gm. lots of tissue were weighed out in 500 ml. Erlenmeyer flasks, to which 1 gm. of calcium carbonate had been added. Two such lots were weighed out for the sugar determinations from each bag of bulbs sampled. These lots were immediately covered with 200 ml. of 95% alcohol and the samples then boiled gently on a steam bath for 5 minutes. When cool they were stoppered, sealed with paraffin, and stored for analysis.

The samples for acid hydrolyzable substances and moisture were prepared as for the 1931 work.

1933 Anatomical Procedure

In 1933 on each sampling date only 5 bulbs were dissected to preserve the developing flower for study. The 1931 work had indicated that the flower development was so uniform in different bulbs under the same conditions that 5 would be sufficient. After being held in 80°F. storage from July 21 to September 5 some samples were removed and placed in 59°, 50°, and 40° F. storage.

Forcing Procedure

In the 1931 investigation two bags of 40 bulbs each were set aside for a study of the forcing responses. These received the same treatment as the two large groups of bulbs; that is, one bag was kept at 80° F. for the entire season while the other bag was transferred from 80° to 50° F. on September 2. On October 29 these two bags of bulbs were planted in flats in a sandy loam. They were rooted in a cool dark bulb storage cellar and in late December brought into a greenhouse with a day temperature of 55-60° F. and a night temperature of 50-55 degrees F. They were kept in this greenhouse until they flowered.

In 1933, lots of 20 bulbs were used for the forcing study. These were given the same storage treatments as the other bulbs in 1933. As there were four different temperature conditions studied in this year there were four lots of 20 bulbs each. These bulbs were planted on October 30 and rooted and forced as described in the 1931 work except that they were not brought into the greenhouse for forcing until January 6.

On March 5, 1934, after most of the flowers on the forced bulbs were past, some of the bulbs were dug, the outer scales removed and the central portion examined. This was done to observe the new growth region, which would have produced leaves and possibly flowers in 1935.

RESULTS

In order clearly to set forth the effects produced by different storage temperatures and to give a foundation on which to discuss the anatomical and chemical changes which undoubtedly underlie or are reflected in the responses noted, the results of forcing the bulbs will be presented first. For convenience the bulbs from the different storage temperatures will be referred to as 80°, 59°, 50°, and 40° bulbs.

Forcing Response

In 1931 the 80° and 50° bulbs used for forcing bloomed in January 1932. The 50° bulbs began to blossom January 12, while fourteen days later the 80° bulbs first started to bloom. Check bulbs used for comparison, which were given only common bulb-house storage but the same cultural conditions otherwise, began to flower on January 22.

In 1933 forced bulbs from the four temperature conditions in storage exhibited differences in growth responses when they were brought from the rooting cellar and placed in the greenhouse. This difference was even more pronounced nine days later when measurements were made. The 80°, 59°, 50°, and 40° bulbs had foliage projecting from the noses of the bulbs 1-2, 2-3, 7-10, and 7-10 inches respectively.

21.

On January 26, 1934, photographs were taken showing the conditions of the four lots of bulbs (see Figs. 19 and 20). At that time the 80°, 59°, 50°, and 40° bulbs had foliage 3-4, 8-10, 14-16, and 12-20 inches long respectively. The 80° bulbs revealed flower buds when the foliage was separated. The 59° bulbs showed flower buds mostly above the foliage. The 50° bulbs had started to blossom on January 24 and on January 26 were nearly in full bloom. The blossoms at that time were 14-16 inches tall. The 40° bulbs despite their luxuriant foliage had only two flowers in bloom. There were no signs of buds in the other eighteen of these twenty bulbs.

Table 1 gives a resumé of the responses from the various lots of bulbs to the forcing treatment given the 1933 bulbs. This table shows that the 80° bulbs were in full bloom on March 1, the 59° bulbs on February 12, while the 50° and 40° bulbs reached full bloom on January 31. The heights of foliage for the 80°, 59°, 50°, and 40° bulbs were 10-12, 12-20, 14-18, and 16-20 inches respectively, while heights of flowers were 12-14, 20-24, 16-20, and 18 inches respectively. The number of flowers blossoming in each lot of 20 bulbs was 31, 34, 32, and 2 blooms for the 80°, 59°, 50°, and 40° bulbs respectively.

Table 1.

1933 Bulbs

Forcing Response

Bulbs (temperature treatment.) ° F.	Date of Full Bloom 1934	Ht. of Foliage Inches	Ht. of Flowers Inches	Number of Flowers
80°-80°	Mar. 1	10-12	12-14	31
80°-59°	Feb. 12	12-20	20-24	34
80°-50°	Jan. 31	14-18	16-20	32
80°-40°	Jan. 31	16-20	18	2

Comparison of Table 1 with Figures 19 and 20 will show the outstanding results of the different temperature treatments of the bulbs during storage on the forcing response. The 50° and 40° bulbs bloomed earliest; but, while the twenty bulbs of the former produced thirty-two flowers, those of the latter produced only two. The foliage on both lots was healthy and adequate although somewhat shorter in the 50° bulbs than in the 40° bulbs. The flower heights averaged the same.

The 59° bulbs blossomed later than those above named but before the 80° bulbs. The foliage was highest of all the lots on the 59° bulbs, while on the 80° bulbs the foliage was shortest. This same relationship held for the heights of flowers to a decided extent.

Figure 20 shows a photograph of the new vegetative buds which were located adjacent to the bases of the scapes of the flowers. The illustration shows the condition of these buds on March 5, 1934, just after the flowers on the bulbs had passed. The scapes adjoining all the buds were removed in the dissection except that of the representative of the 40° bulbs. Here the dead flower, which never elongated, can be seen on the top of the scape. This condition was noted in eighteen of the twenty bulbs under the 40°F. condition.

Anatomical Development

Figure 2 shows the condition of a flower in a bulb in the 1931 investigation. The flower was in this state of development on July 1, the day after the bulbs were dug from the field. Figure 3 shows the state of the flower in a bulb on August 31, 1931. It had been stored since July 21 at 80° F. Figure 4 shows the flower of an 80° bulb as it appeared on October 12. Figure 5 shows a flower from a 50° bulb also on October 12.

Figures 7 through 12 show the anatomical development of the buds during the period of storage at the various temperatures in 1933. Figure 7 shows the condition of a bud on July 24 at 80°F., just after the bulbs had been placed in storage. Figure 8 shows the flower of an 80° bulb on September 5. Figure 9 shows how the flower of an 80° bulb appeared on October 30, while Figures 10, 11, and 12 show how the flowers of bulbs looked in 59°, 50°, and 40° bulbs also on October 30.

From the study of a large number of buds from other bulbs it is believed that the sections illustrated are representative of all the flowers in bulbs under the same experimental conditions.

Figures 13 through 18 give further evidence, of a cytological nature; they show the varying degree of development of the anthers in the flowers of the 1933 bulbs at various temperatures. In the killing and fixing of the narcissus buds, as well as in the staining of the sections, the aim was to prepare sections for gross anatomical study only. The sections, however, showed such striking evidences of a cytological nature that photomicrographs were made of details of the anthers of the developing flowers.

Figure 13 illustrates sporogenous tissue in an anther of the flower bud from a bulb on July 24 at the beginning of the storage period at controlled temperatures. Figure 14 shows a similar view, but on September 5, after the bulbs had been at 80°F. for about six weeks. Figure 15 shows sporogenous tissue still in the anther of a flower bud of an 80° bulb on October 30. However, Figure 16, which is from an anther of the flower of a 59° bulb, shows that on the same date, October 30, young pollen grains had formed. Figure 17 illustrates development of pollen in the 50° bulbs and shows that the microspores are just breaking away from the tetrads after the second division of the pollen mother cells. Figure 18 shows the condition within the 40° bulbs on October 30; here the pollen mother cells are in the prophase of the first division.

Study of the photomicrographs of the flower buds for the two years will disclose the development of the flowers in the bulbs stored under the different temperature conditions. These illustrations reveal that at the end of the storage period the flowers in the 80° bulbs had developed least, followed by those in the 40° bulbs. The flowers of the 50° bulbs are shown to have elongated twice as much as the 80° bulbs, while the 59° bulbs show the longest flowers of all, as shown by Figure 10. The photomicrographs, Figures 13 through 18, of the anther development of the bulbs' flowers show the same relationship regarding flower development as is shown in the anatomical illustrations. Study of all these photomicrographs will reveal that anatomical or cytological development of the flowers at the end of the storage period is not, in this experiment, an index of early flowering response.

Biochemical Results

Reference to Tables 2 through 5 will show the percentages of weight, moisture content, acid-hydrolyzable substances, sucrose, and reducing sugars on the various sampling dates as indicated in 1931 and 1933. With the exception of the weight figures, which are for whole bulbs, all the above are based on 100 gms. of living bulb tissue at the time the samples were preserved in alcohol for analysis.

Table 2. 1931 Shrinkage

Shrinkage of Narcissus Bulbs at Different Dates

During Storage on a Basis of 100 gms. of Bulbs on July 22.

<u>Dates</u>	<u>"80° bulbs"</u>	<u>"50° bulbs"</u>
July 22	100	-
Aug. 5	95.19	-
" 19	92.23	-
Sept. 2	89.56	-
" 16	86.49	88.37
" 30	83.49	87.53
Oct. 14	80.26	85.73
" 28	78.32	83.73

Table 3. 1933 Shrinkage

Shrinkage of Narcissus Bulbs at Different Dates

During Storage on a Basis of 100 gms. of Bulbs on July 24.

<u>Dates</u>	<u>"80° bulbs"</u>	<u>"59° bulbs"</u>	<u>"50° bulbs"</u>	<u>"40° bulbs"</u>
July 24	100	-	-	-
Aug. 14	95.98	-	-	-
Sept. 5	94.83	-	-	-
Oct. 2	92.43	90.87	92.81	93.56
" 30	88.89	88.18	91.60	93.02

Table 4.

1931 Bulbs

Moisture Content, Acid Hydrolyzable Substances, Sucrose and Reducing Sugars in 100 gms.

of Bulb Tissue at the time Samples were Preserved for Analysis.

Date	Moisture Content		Acid-hydrolyzable Substances (as Dextrose)		Sucrose (as Invert Sugar)		Reducing Sugars (As invert sugar in presence of sucrose)	
	"80°bulbs"	"50°bulbs"	"80°bulbs"	"50°bulbs"	"80°bulbs"	"50°bulbs"	"80°bulbs"	"50°bulbs"
July 23	66.72	-	25.48	-	2.77	-	0.43	-
Aug. 6	66.46	-	25.50	-	3.22	-	0.49	-
" 20	66.28	-	25.48	-	3.36	-	0.40	-
Sept. 3	66.28	-	25.39	-	3.36	-	0.36	-
" 17	66.42	66.02	25.29	25.31	3.54	3.59	0.28	0.60
Oct. 1	66.22	65.86	25.52	24.57	3.64	4.24	0.29	0.59
" 15	66.12	65.61	25.62	24.53	3.60	4.54	0.29	0.60
" 29	65.94	65.21	25.67	24.88	3.77	4.66	0.32	0.59

Table 5.

1933 Bulbs

Moisture Content, Acid hydrolyzable Substances, Sucrose and Reducing Sugars in 100 gms.
of Bulb Tissue at the time Samples were Preserved for Analysis.

Dates	Moisture Content				Acid Hydrolyzable Substances (as Dextrose)				Sucrose (as invert sugar)				Reducing sugars (As invert sugar in presence of sucrose)			
	Bulbs at (Fahrenheit)				Bulbs at (Fahrenheit)				Bulbs at (Fahrenheit)				Bulbs at (Fahrenheit)			
	80°	59°	50°	40°	80°	59°	50°	40°	80°	59°	50°	40°	80°	59°	50°	40°
July 25	65.72	-	-	-	26.87	-	-	-	2.33	-	-	-	0.60	-	-	-
Aug. 15	65.56	-	-	-	26.10	-	-	-	2.82	-	-	-	0.57	-	-	-
Sept. 6	65.09	-	-	-	25.75	-	-	-	3.08	-	-	-	0.49	-	-	-
Oct. 3	65.47	65.36	65.02	65.08	25.32	25.65	24.67	24.37	2.75	3.58	4.04	3.84	0.57	0.60	0.83	0.93
" 31	65.27	63.82	64.93	65.32	24.87	26.15	24.45	23.82	3.02	4.34	4.61	4.64	0.64	0.47	0.80	1.08

Table 2 shows the shrinkage of the bulbs during the period of storage in 1931. These figures are based upon 100 gms. of bulbs at the time they were put into storage at 80°F. This table indicates that the 80° bulbs lost 21.68% of their weight during the period of storage, while the 50° bulbs lost but 16.27%. Table 3, for the 1933 bulbs, discloses that in this year during the storage period the 80° bulbs lost 11.11%, the 59° bulbs 11.82%, the 50° bulbs 8.40%, and the 40° bulbs but 6.98%.

Table 4 shows the changes in the moisture content of the 1931 bulbs during the storage period. This content was 66.72% at the beginning of the period at 80°F. storage; in the 80° bulbs this varied but little and at the end of the storage period was 65.94%. The 50° bulbs show a slight decrease in moisture content below the 80° bulbs and at the end of the season show a content of 65.21%. Table 4 further shows the changes in the acid-hydrolyzable substances, computed as dextrose, in the 80° and 50° bulbs. In the former this content did not change appreciably during the season. On July 23 the bulbs contained 25.48%, while on October 29 they showed 25.67%. Also in Table 4 the sucrose contents of the 1931 bulbs are shown. On July 23 the 80° bulbs contained 2.77% which increased by the end of the season to 3.77%. The 50° bulbs, however, showed a greater increase in

sucrose, and the analyses on October 29 showed a content of 4.66%. Finally Table 4 shows the percentage of reducing sugars in the 1931 bulbs. The 80° bulbs contained 0.43% of reducing sugars at the beginning of the storage period. This amount decreased until September 17, and then increased to 0.32% by October 29. The 50° bulbs showed a steady content between 0.59% and 0.60% during their period at 50°F.

Table 5 shows the changes in moisture content, acid-hydrolyzable substances, sucrose, and reducing sugars of the bulbs under the conditions of the experiment in 1933. In the 80° bulbs the moisture content varied less than one per cent during storage. In the 59° bulbs this content showed a slight increase as the season progressed, followed by a decided drop. In the 50° bulbs there was a slight decline, while in the 40° bulbs a slight increase occurred.

The acid-hydrolyzable substances showed a gradual decline from 26.87% in the 80° bulbs, while in the 59° bulbs a drop was followed by a slight increase at the end of the season. In both the 50° bulbs and the 40° bulbs there was a decline in these acid-hydrolyzable substances as the season progressed. On the last sampling day the acid-hydrolyzable contents of these bulbs were 24.45% and 23.82% respectively.

Sucrose in the 80° bulbs on July 25 was 2.33%. The content varied slightly during the season and at the end was 3.02%. The sucrose content increased in the 59°, 50°, and 40° bulbs and on October 31 was 4.34%, 4.61%, and 4.64% respectively.

The percentage of reducing sugars in the bulbs at 80°F. started at 0.60%, dropped to 0.49% on September 6, and then increased to the end of the season, when it was 0.64%. This content showed a distinct increase in the 59° bulbs after they were changed from 80°F. to their designated temperature. However, this content again dropped on October 31 to 0.47%. The 50° bulbs likewise rose to 0.83% and then dropped slightly to 0.80% on October 31, while the 40° bulbs continuously increased in reducing sugars as their storage period progressed; the last analysis showed this content to be up to 1.08%.

Figures 1 and 6 show curves for the changes in weight, and moisture, acid-hydrolyzable substances, sucrose, and reducing sugars contents for the entire periods of storage of the bulbs in 1931 and 1933 respectively.

It will be noted that in 1931 the average shrinkage of the bulbs during storage was about 20%, while in 1933 the shrinkage centered around 10%. In both cases the shrinkage was quite gradual through the season. The temperatures during the latter parts of the seasons did not seem to have any great effects on the shrinkage.

The moisture contents, which were calculated from weights of living bulb tissue, did not show any appreciable change during the storage period in either year of sampling. In 1931 the losses at the end of the season were on either side of one per cent, while in 1933 all the lots lost less than one per cent except the 59° bulbs, which lost about 2%. The 59° curve dropped from the curves of the other lots between October 3 and 31. The loss of moisture in these 59° bulbs may be of significance, although in this connection it must be stated that these bulbs had to be kept in a small , cold storage compartment in a steam-heated building where the relative humidity was low during October after the steam was turned on.

Figures 1 and 6 also show that in general the curves of acid-hydrolyzable substances dropped as the storage seasons continued. The 80° bulbs in 1931 and the 59° bulbs in 1933, however, show slight increases later in the season. The increase of these substances in the 59° bulbs and the decided decrease in the 40° bulbs in 1933 are noteworthy in the light of anatomical and forcing behaviors which will be discussed later in this paper.

All the curves for the sucrose contents of the bulbs show increases as the storage season is lengthened, except on October 3, 1933, when a slight drop in the sucrose content occurred in the 30° bulbs. Here again it is of particular interest to note that the 40° bulbs show a high sucrose content comparable with the 50° bulbs. Finally, in the two graphs the curves for reducing sugars show increases as the season progressed with the exception of the 59° and 50° bulbs at the last sampling date in 1933, and, more emphatically, the 50° bulbs in 1931. These did not show an appreciable increase or decrease after September 17. As in the case of the sucrose contents the reducing sugars fractions in the 40° and 50° bulbs remained relatively high at the end of the storage season. This is of interest and will be considered under the discussion.

DISCUSSION

These investigations have shown noteworthy differences in the forcing responses of bulbs held at different storage temperatures. The anatomical and chemical changes occurring in the bulbs during the storage period were no less remarkable. It is of especial interest to note the relationships obtaining between the forcing responses and the anatomical and chemical changes occurring during storage. The evidence shows that the 50° bulbs gave the earliest satisfactory flowering response. However, the anatomical and cytological evidence show that the 59° bulbs were furthest advanced at the end of the storage period. The 40° bulbs, which were decidedly less advanced anatomically than the 59° bulbs, bloomed with the 50° bulbs, fifteen days earlier than the 59° bulbs. Only two of the twenty 40° bulbs, however, flowered. The 80° bulbs, which were least advanced at the end of the storage period, flowered last and were the least vigorous.

Detailed consideration of each temperature group of bulbs will amplify the above statements. As has already been pointed out, the 50° bulbs flowered earliest in spite of the greater development of the 59° bulbs at the end of the storage period as shown by Figures 10, 11, 16 and 17. This forging ahead of the 50° bulbs bears out in part the findings of Blaauw et al. (3) that the developing flowers

and foliage of bulbs in storage at 13° C. (55° F.) progress most rapidly in July and August, but that bulbs which are held at a lower temperature, 9° C. (48° F.) during this same period will flower earlier.

The photographs of the bulbs at 40° F. show that but two of the 20 bulbs flowered; nevertheless none of the buds preserved on October 30 for anatomical study showed signs of death at that time (see figs. 12 and 18). Apparently death took place during the period of rooting or forcing. The flowers of these bulbs could not respond to the conditions of rooting and forcing which were favorable to the flowers in the other bulbs.

The 80° bulbs responded slowly to their temperature treatment. The photomicrographs for the 1931 and 1933 work show a slow but continuous growth and development throughout the storage seasons, but there was not the activation as noted in the bulbs placed at lower temperatures either in the development during storage or in the ultimate forcing response.

Of interest are the photomicrographs showing the development of the anthers in the flowers of the bulbs held in storage at the various temperatures. These illustrations show the anther tissue in various stages of development from sporogenous tissue to young pollen grains. The stage of the development of these anthers is directly correlated with the size of the flower buds in all cases. It is remarkable that pollen grains should

appear in the developing flowers more than three months before the flowers bloom. However, in narcissus, at least, this seems to be the general rule. Nagao in Japan (25) reported that for Narcissus pseudonarcissus growing out-of-doors at Kyoto, Japan, the time to find the reduction division in the pollen mother cells is from the middle of October to early November.

This investigation, so far as the anatomical studies are concerned, proves that, using as an index only the length of the flower within the bulb during the storage season, the time that a narcissus bulb will flower after storage cannot be predicted. The anatomical and cytological evidence corroborates the findings of Lumsden (23), van Slogteren (31), and Huisman (22) that narcissus bulbs are developing and growing within themselves while they are in summer storage. As the writer (23) has pointed out, this development precludes reference to narcissus bulbs as dormant or in a rest period during the summer if the terms "dormant" and "rest period" are construed to refer to conditions in the bulbs where no growth takes place. Such a conception of dormancy is stated by Howard (21) and implied by Miller (24).

The noteworthy biochemical changes which occurred in bulbs during storage throw further light on the anatomical and forcing behavior. These changes may not be causal, but at least they are indicators of physiological activity.

Blaauw et al. (3) postulated that in forcing narcissus a low temperature (between 55 and 48 degrees F.) may be necessary for the bulbs to make essential chemical conversions. The graphs (Figs. 1 and 6) show that hydrolysis measured by acid-hydrolyzable substances proceeded more rapidly in the 50° bulbs than in the 80° bulbs. The anatomical and forcing evidence shows that the 50° bulbs developed more rapidly and flowered earlier. The graphs show further that the 50° bulbs contained more available food as sucrose and reducing sugars than did the 80° bulbs.

The similarity of the 1931 and 1933 results is salient and indicates a striking difference in the physiological activity of the 80° and 50° bulbs.

The 59° bulbs showed changes which were not consistent with those which occurred in the 80° or 50° bulbs. Acid-hydrolyzable substances decreased in the 59° bulbs until October 3 and then showed an increase again at the end of the season. Their sucrose content at the end of the season was relatively high but their reducing sugars content relatively low. However, at no point in the storage season were the amounts of sucrose or reducing sugars in the 59° bulbs equal to the amounts found in the 50° bulbs; yet at the end of the storage season the photomicrographs show the 59° bulbs to have developed and elongated more than the 50° bulbs.

The analyses of the 80° bulbs give further evidence that an abundance of reducing sugars is not correlated with rapid growth, for the 80° bulbs showed only slightly less reducing sugars than the 59° bulbs on October 3 and more on October 31.

The 40° bulbs are striking in demonstrating that the content of available sugars is not a direct index of growth activity in narcissus bulbs. These 40° bulbs through the season hydrolyzed more polysaccharides, had more reducing sugars, and contained nearly as much sucrose or more than any of the bulbs at the other temperatures; yet the development and growth of their flowers while in storage were not equal to either the 59° or 50° bulbs.

The flowers of the 40° bulbs, however, forced as early as the 50° bulbs, but only two flowers developed from the twenty bulbs. The death of the flowers in eighteen out of twenty bulbs which had been at 40°F. would appear to be associated with a high available sugar content which could not be utilized for flower development at the low temperature or under the rooting and forcing conditions. It is conceivable that the low temperature either destroyed or rendered inactive enzymes or some growth-promoting substance and this ultimately resulted in the death of the flowers. It must be borne in mind, however, that the foliage of the bulbs was not affected. In this connection it has been noted that any adverse conditions to narcissus bulbs usually are first noticed in injury or death to the

developing flower.

Further studies on the effect of the 40° temperature are suggested. It is conceivable that a 40° temperature for a shorter time in storage followed by a higher temperature more suited to elongation would give a combination capable of forcing narcissus bulbs in the minimum length of time without flower injury.

From the evidence on carbohydrates as shown in Figure 6 it would appear that the sucrose content in the bulbs at the end of the storage period is an index of early flowering. The 50° and 40° bulbs had the highest sucrose contents and flowered first, followed by the 59° bulbs, which had less sucrose; the 80° bulbs flowered last, having had the lowest sucrose content at the end of the storage period. It must also be borne in mind that the 40° bulbs on the other hand produced only two flowers from twenty bulbs.

The 1931 work as indicated by Figure 1 bears out this correlation between sucrose content and early flowering. Hasselbring's paper (19) also shows a high sucrose content of narcissus bulbs at the end of storage correlated with greater activity within the bulbs.

The 59° bulbs showed the greatest growth during the storage period. It will be recalled that their outstanding loss of moisture might be due to the fact that the relative humidity in the place where they were stored was low between October 3 and 31. Correction for this extreme moisture loss

would straighten out the curve for acid-hydrolyzable substances and practically eliminate the apparent rise as shown for these bulbs at the end of the storage season. This correction would affect the sucrose and reducing sugars contents, making them lower, but only to a slight degree. But the reducing sugars content of the 59° bulbs on October 31 is already lowest of all the bulbs. It may be possible that the 59° bulbs at the end of the storage period could not convert stored food available sufficiently fast adequately to accommodate the large amount of meristematic tissue contained in their elongating flowers as shown by Figure 10.

It is noteworthy that the moisture contents of the living bulb tissue in both 1931 and 1933 remained so nearly constant throughout the storage season. While the whole bulbs in 1931 lost about 10% of their weight and those in 1933 lost around 20%, yet for the two years the moisture content of the living tissue was held within close limits. The work of Hasselbring (19) in 1929 also shows the consistency of this moisture content for the live tissue of the bulbs during storage.

The ability to maintain a rather steady moisture content suggests a consideration of bound and free water in the bulbs to determine if the former is related to this ability to resist water loss. The highly hygroscopic nature of the bulb tissue which was noted in determining the dry weight of the tissue also supports such a supposition.

A further consideration of the moisture content suggests the possibility that the rapid growth organization of the 59° bulbs permitted a releasing of the bound water in the bulbs. The drop in the moisture content curve at the end of the season might then be due more to this phenomenon than to the fact that the relative humidity was low in the storage chamber.

Because bulbs are constantly growing in storage it is difficult to consider an equilibrium of acid-hydrolyzable substances, sucrose, and reducing sugars at any given storage temperature. It is probable that the constant increase in meristematic tissue would cause a constant change in this equilibrium although the temperature remained the same. Denny (5) has suggested the existence of a starch-sucrose equilibrium in working with potato tubers in storage but questions whether the relationship is direct. Narcissus bulbs, however, show much more growth activity in storage than potatoes in storage.

Biochemical evidence, together with anatomical and forcing responses, suggests that further studies of storage conditions would yield valuable physiological information and practical applications. It remains to be determined whether a shorter 40° F. storage temperature, which has been shown not to be favorable for rapid growth within the bulb, may not induce an even more rapid flowering

response and at the same time avoid the death of the blossoms.

If such should be the case the practical applications of value to florists are apparent.

SUMMARY

Bulbs of King Alfred narcissus (Narcissus pseudonarcissus L.) were dug from the field in the early summer of 1931 and another lot in 1933. These were dried and placed in storage at 80° F. Near the middle of the storage period the bulbs were divided into lots; in 1931 equal numbers were continued at 80° F. and placed at 50° F.; in 1933 equal numbers were continued at 80° F., placed at 59, 50, and 40 degrees F. The bulbs are spoken of as 80° bulbs, 59° bulbs, etc., depending upon the temperature to which they were subjected during the second half of their storage period.

During the time the bulbs were in storage, between late July and late October, samples of the bulbs were taken at intervals to study carbohydrate and anatomical changes within the bulbs. Some of the bulbs under the storage conditions named were forced at the end of the storage period.

The bulbs in 1931 lost around 20% of their weight while in storage, while in 1933 the loss of weight under the four conditions centered around 10%.

The moisture content of the fleshy parts of the bulbs did not lower more than two per cent under any conditions and in most cases was less than one per cent. Slight increases in these contents were manifest at some points.

The amounts of acid-hydrolyzable substances, sucrose and reducing sugars are calculated on a basis of 100 gms. of bulb

tissue at the time the samples were preserved in alcohol. The acid-hydrolyzable substances in the bulbs showed a loss in general during the storage period. The sucrose contents showed gains with one exception during the same period. The loss was in the 80° bulbs between September 6 and October 3, 1933. The reducing sugars contents of the bulbs likewise showed gains throughout the storage period with few exceptions, notably in the 59° bulbs between October 3, to 31, 1933.

There does not appear to be a correlation between the loss of acid-hydrolyzable substances and gain in sugars with the growth and development of the flowers in the bulbs during storage. The very active 59° bulbs showed less hydrolysis than the relatively inert 80° and 40° bulbs throughout the storage period. In the 40° bulbs growth in storage was notably contrary to hydrolytic processes. After these bulbs had high sucrose and reducing sugars contents the growth continued slowly. Apparently the low temperature inhibited metabolic activity.

The ultimate forcing responses seem to be associated with sucrose contents during the storage season. Under all the conditions of storage in 1931 and 1933 high sucrose content in storage was correlated with early flowering.

Photomicrographs of sections made for anatomical study of the primary buds of the bulbs show that the flowers are already formed in the buds in July for flowering the following spring. These illustrations also show that the differentiation

and growth of the flowers took place in all the lots of bulbs under all the conditions of the experiments and during all the intervals between the times of sampling.

Other photomicrographs showing the anthers within the developing flowers reveal that the growth of the flowers is correlated positively with the development of the pollen grains in the anthers.

In storage the growth was most accelerated in the 59° bulbs, then the 50°, 40°, and 80° bulbs respectively. In the 1933 forcing of the bulbs, however, the 50° and 40° bulbs flowered first, then the 59° bulbs, followed by the 80° bulbs. Yet in the 40° bulbs only two of the twenty bulbs bore blossoms. The balance of the buds were found dead at the centers of the bulbs. This occurrence seemed associated with the high sugar content of the bulbs, coupled with the lack of conditions favorable for elongation. From the above it is apparent that anatomical development in storage is not necessarily an index of forcing responses.

The complete data suggest that a temperature of 40° for a brief period of time in storage followed by a higher temperature around 59° F. may be the optimum temperature for early flowering of narcissus bulbs, the lower temperature to hasten conversions and the higher temperature to promote metabolic activities and elongation.

Figure 1. 1931 Investigations

Chart showing the weight reduction in bulbs during storage; also the moisture, acid-hydrolyzable substances, sucrose, and reducing sugars contents, based on 100 gms. bulb tissue at the time it was preserved in alcohol.

1931

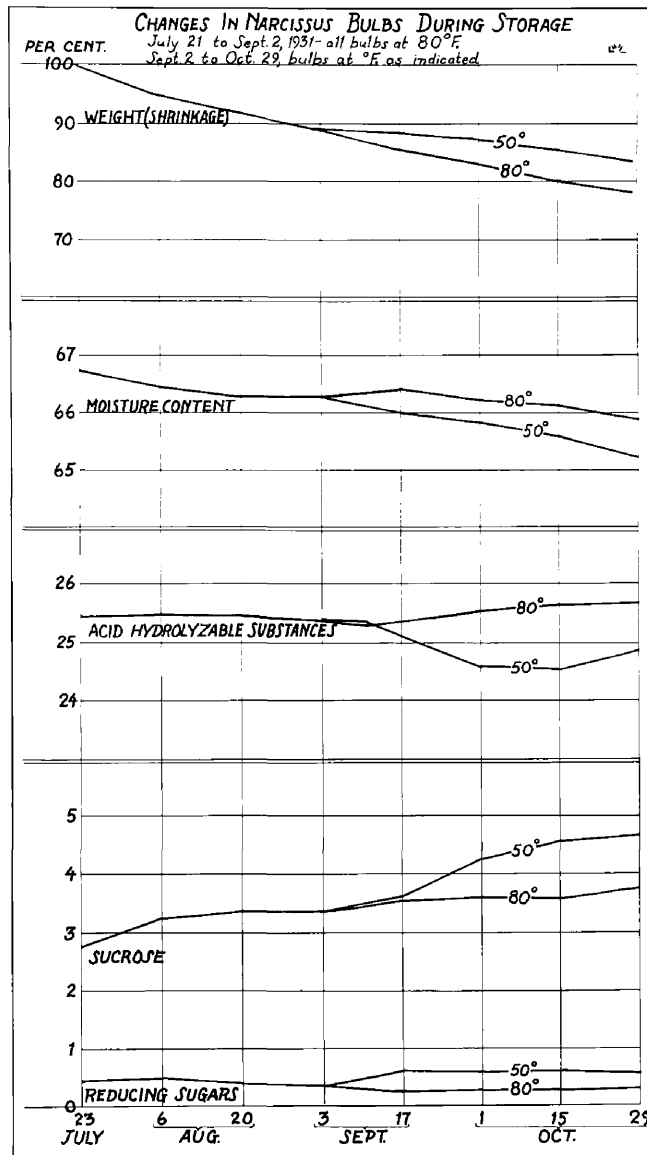


FIG. 1

Figures 2 and 3. 1931 Investigations

Photomicrographs of sections showing the development of flower buds in narcissus bulbs during storage.

Figure 2 is from a bulb on July 1, the day after the bulbs were dug.

Figure 3 is from a bulb on August 31, after the bulb had been in storage at 80° F. since July 21.

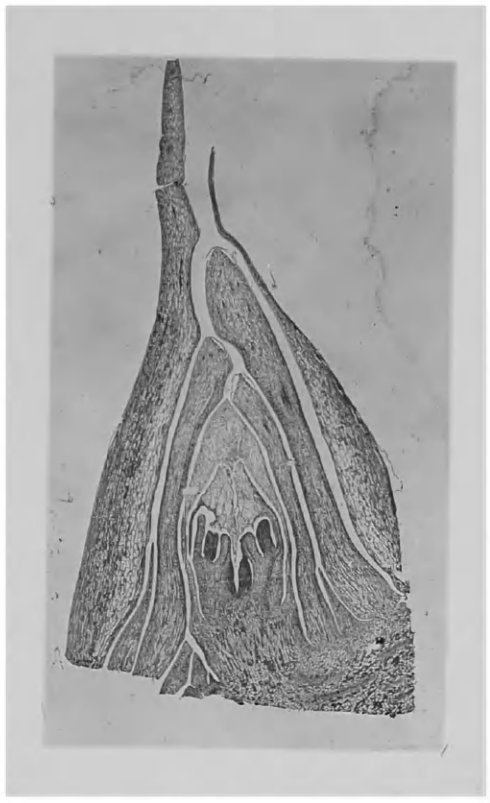


FIG. 2

x 8



FIG. 3

x 8

Figures 4 and 5. 1931 Investigations

Photomicrographs of sections showing the development of flower buds in narcissus bulbs during storage. The illustrations show the conditions in two bulbs on October 12.

Figure 4 is from a bulb which was kept at 80° F. from July 21.

Figure 5 is from a bulb which was transferred from 80° F. to 50° F. on September 2.

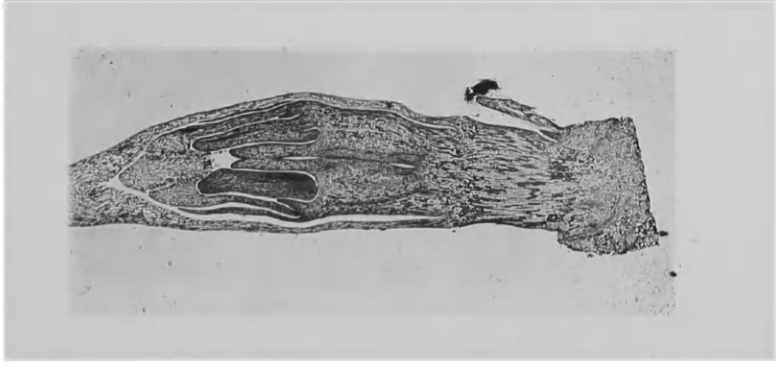


FIG. 4

x8

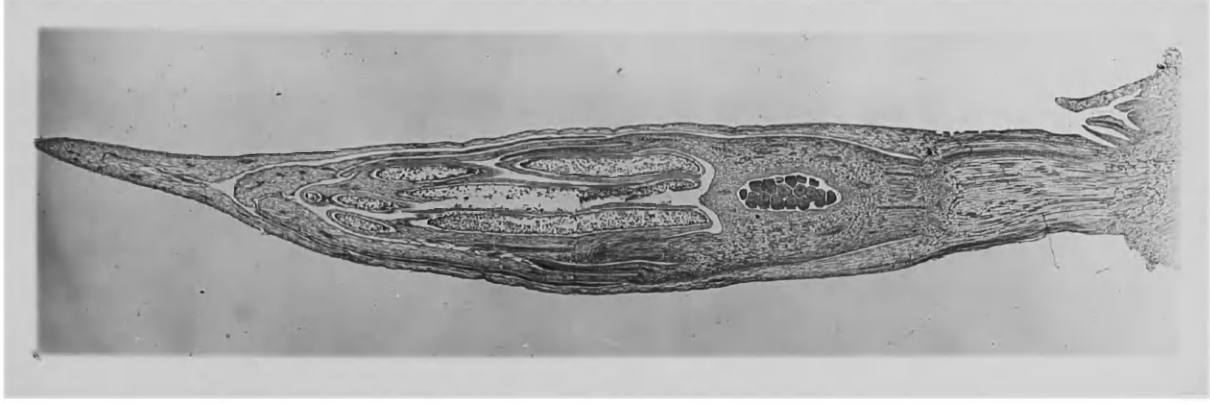


FIG. 5

x8

Figure 6. 1933 Investigations

Chart showing the weight reduction in bulbs during storage; also the moisture, acid-hydrolyzable substances, sucrose, and reducing sugars contents, based on 100 gms. bulb tissue at the time it was preserved in alcohol.

1933

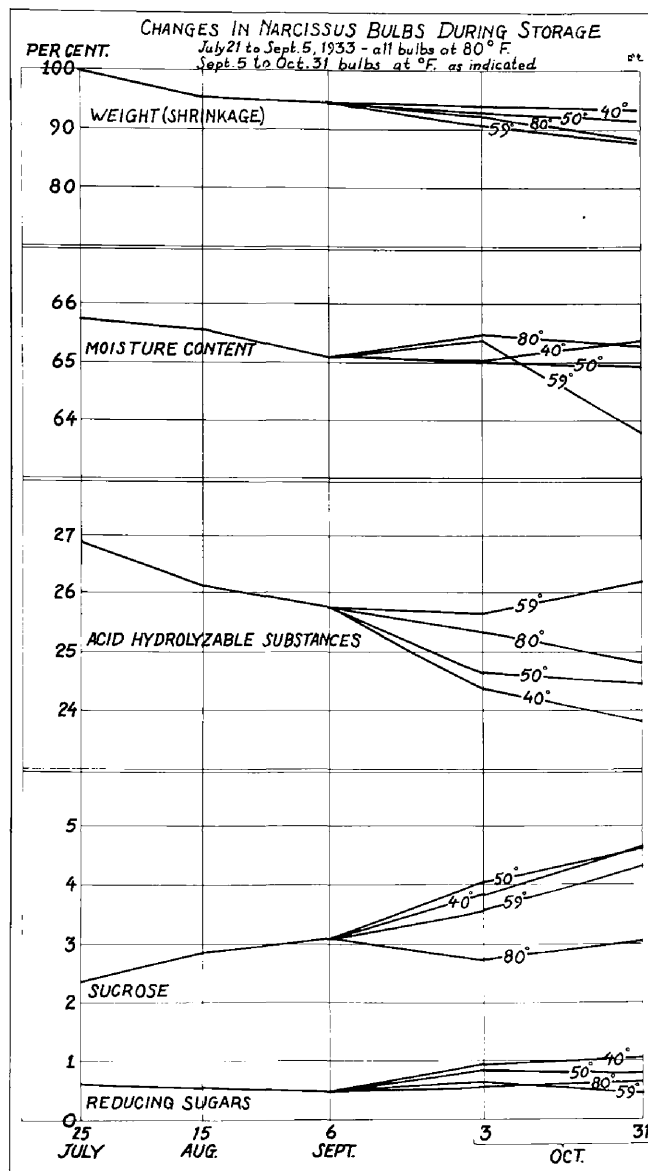


FIG. 6

Figures 7 and 8. 1933 Investigations

Photomicrographs of sections showing the development of flower buds in narcissus bulbs during storage. The figures show the conditions in two bulbs placed in storage at 80° F. on July 21.

Figure 7 is from a bulb on July 24.

Figure 8 is from a bulb on September 5.

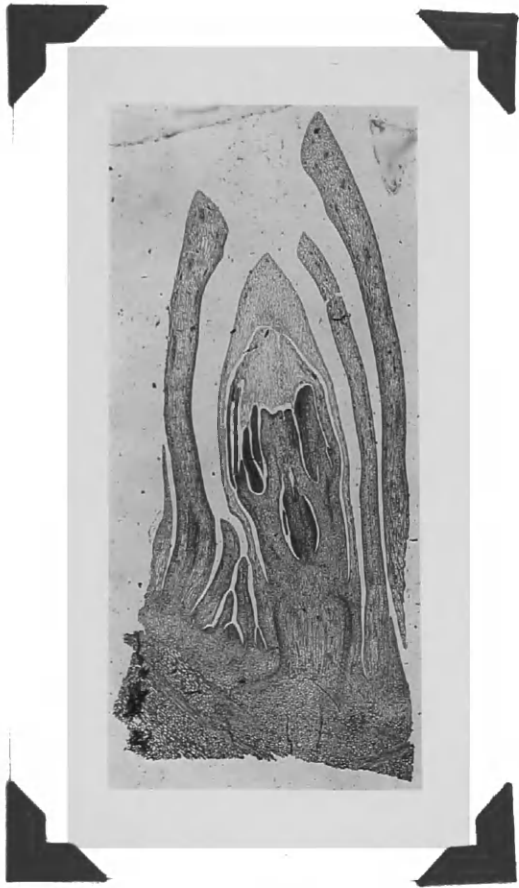


FIG. 7

X8

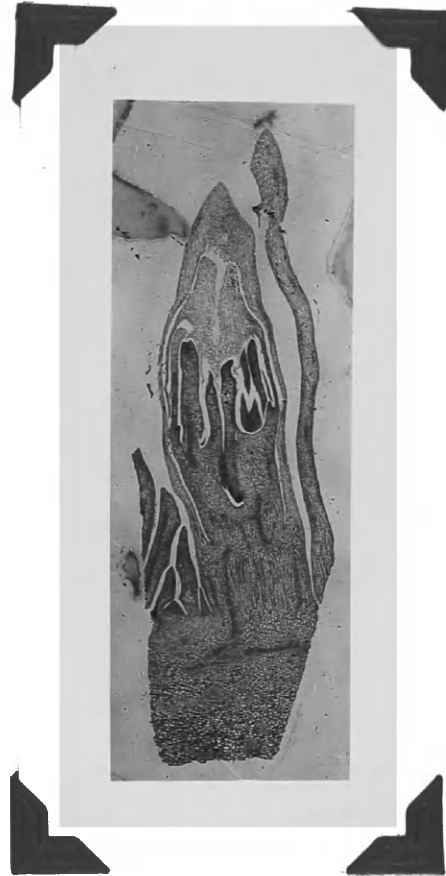


FIG. 8

X8

Figures 9 and 10. 1933 Investigations

Photomicrographs of sections showing the development of flower buds in narcissus bulbs during storage. The illustrations show the conditions in two buds on October 30.

Figure 9 is from a bulb which was kept at 80° F. from July 21.

Figure 10 is from a bulb which was transferred from 80° F. to 59° F. on September 5.

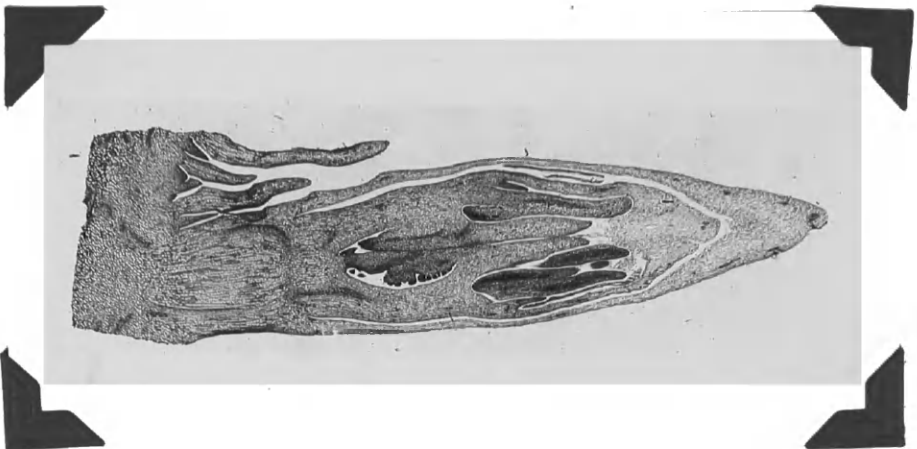


FIG. 9

x 8

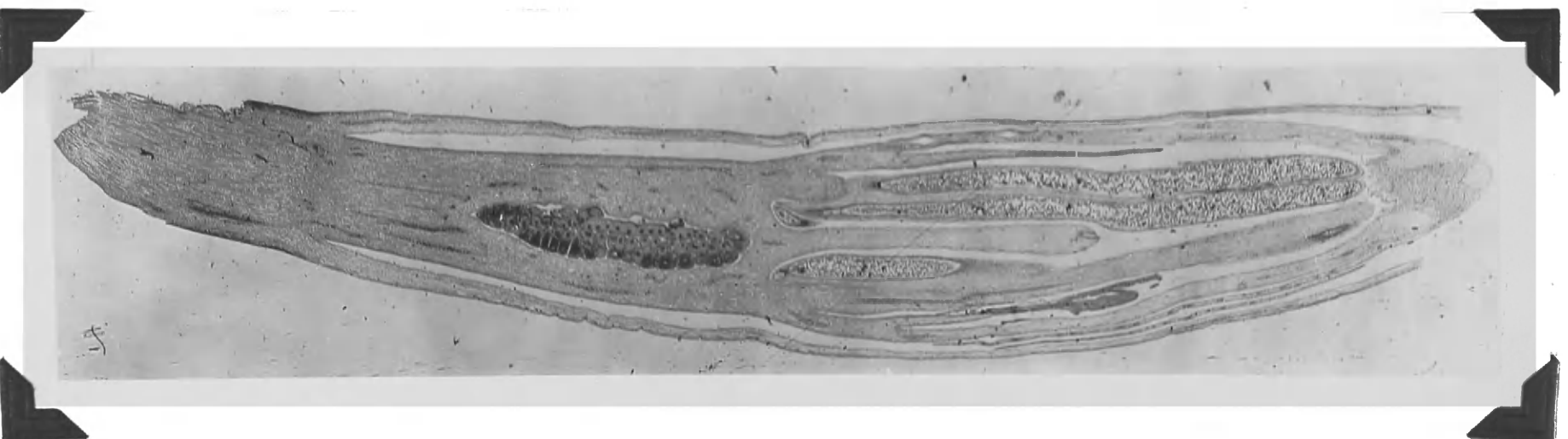


FIG. 10

x 8

Figures 11 and 12. 1933 Investigations

Photomicrographs of sections showing the development of flower buds in narcissus bulbs during storage. The illustrations show the conditions in two buds on October 30.

Figure 11 is from a bulb which was transferred from 80° F. to 50° F. on September 5.

Figure 12 is from a bulb which was transferred from 80° F. to 40° F. on September 5.

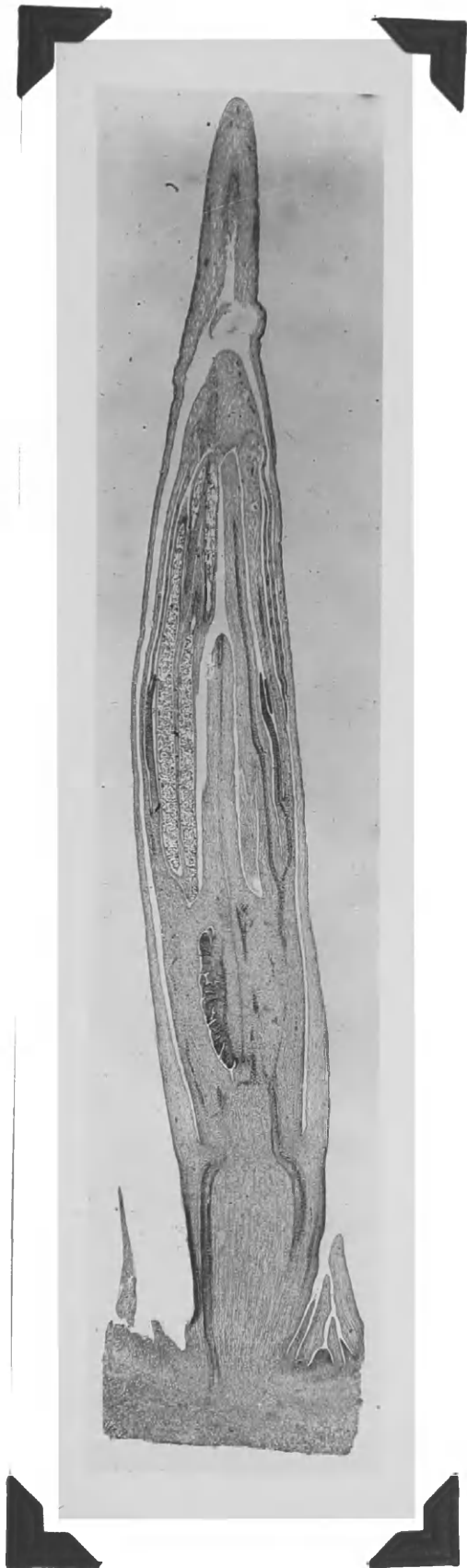


FIG. 11

X 8

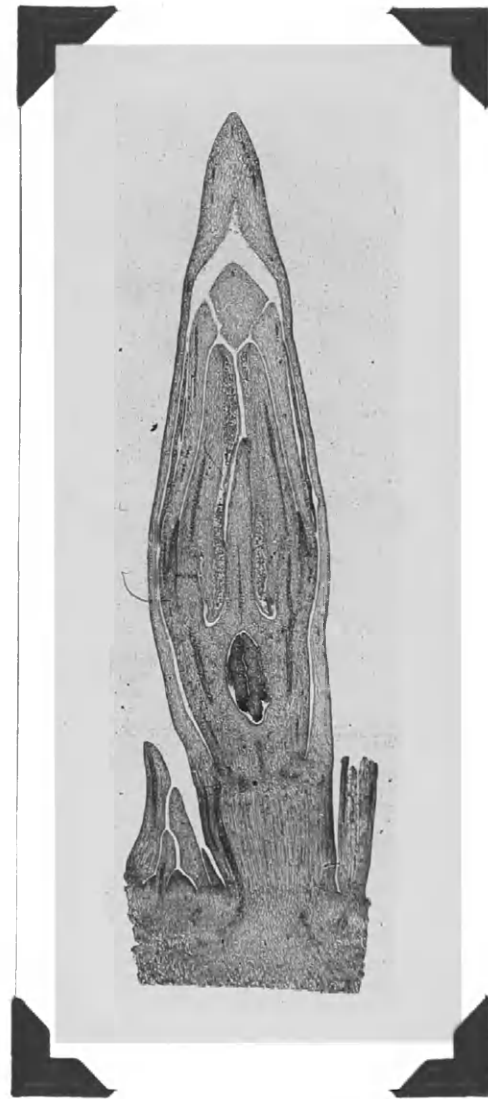


FIG. 12

X 8

Figure 13. 1933 Investigations

**Photomicrograph of a section showing the
sporogenous tissue in the anther of the flower
bud shown in Figure 7.**

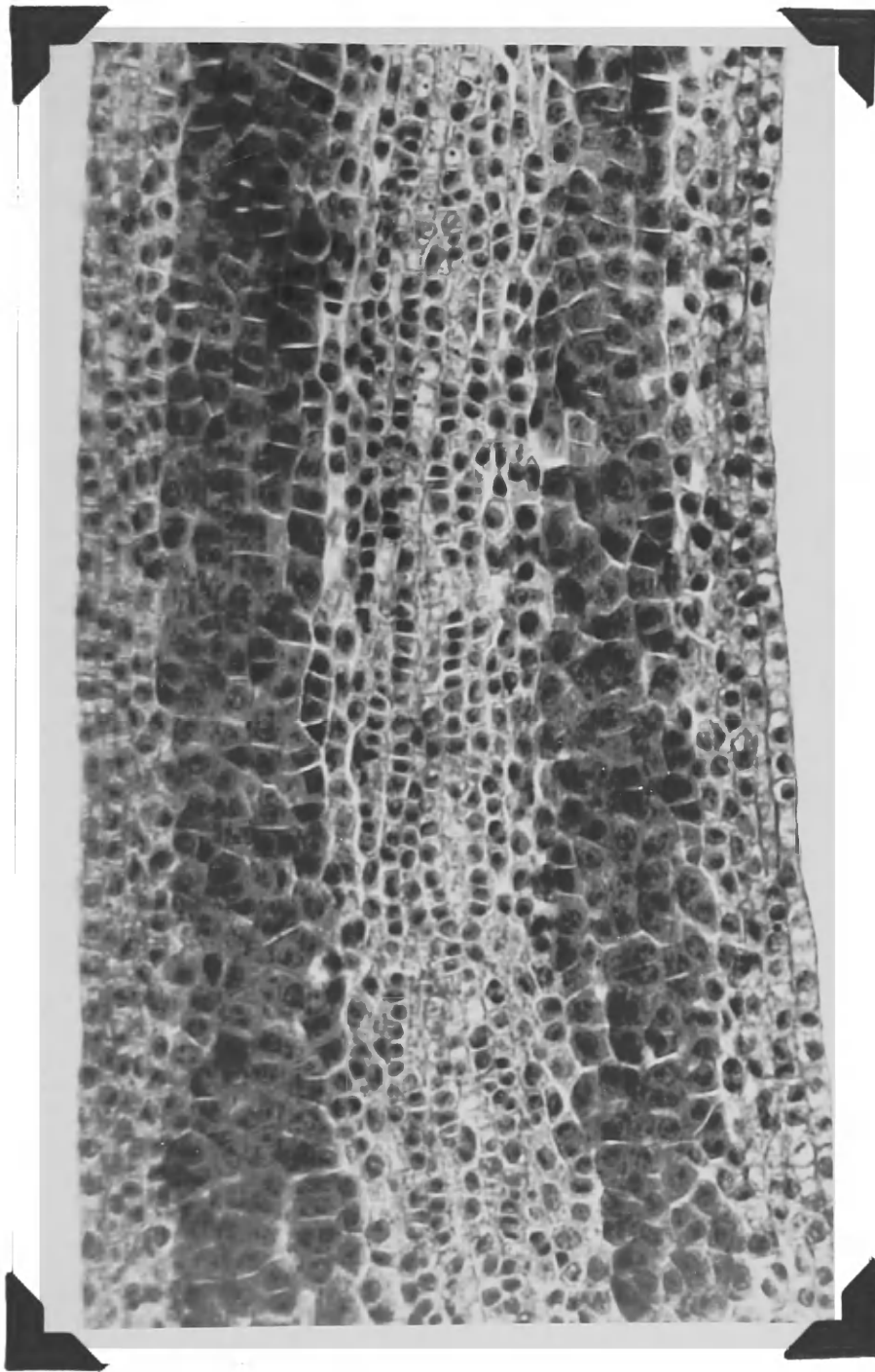


FIG. 13

X300

Figure 14. 1933 Investigations

Photomicrograph of a section showing the
sporeogenous tissue in the anther of the flower
bud shown in Figure 8.

65.

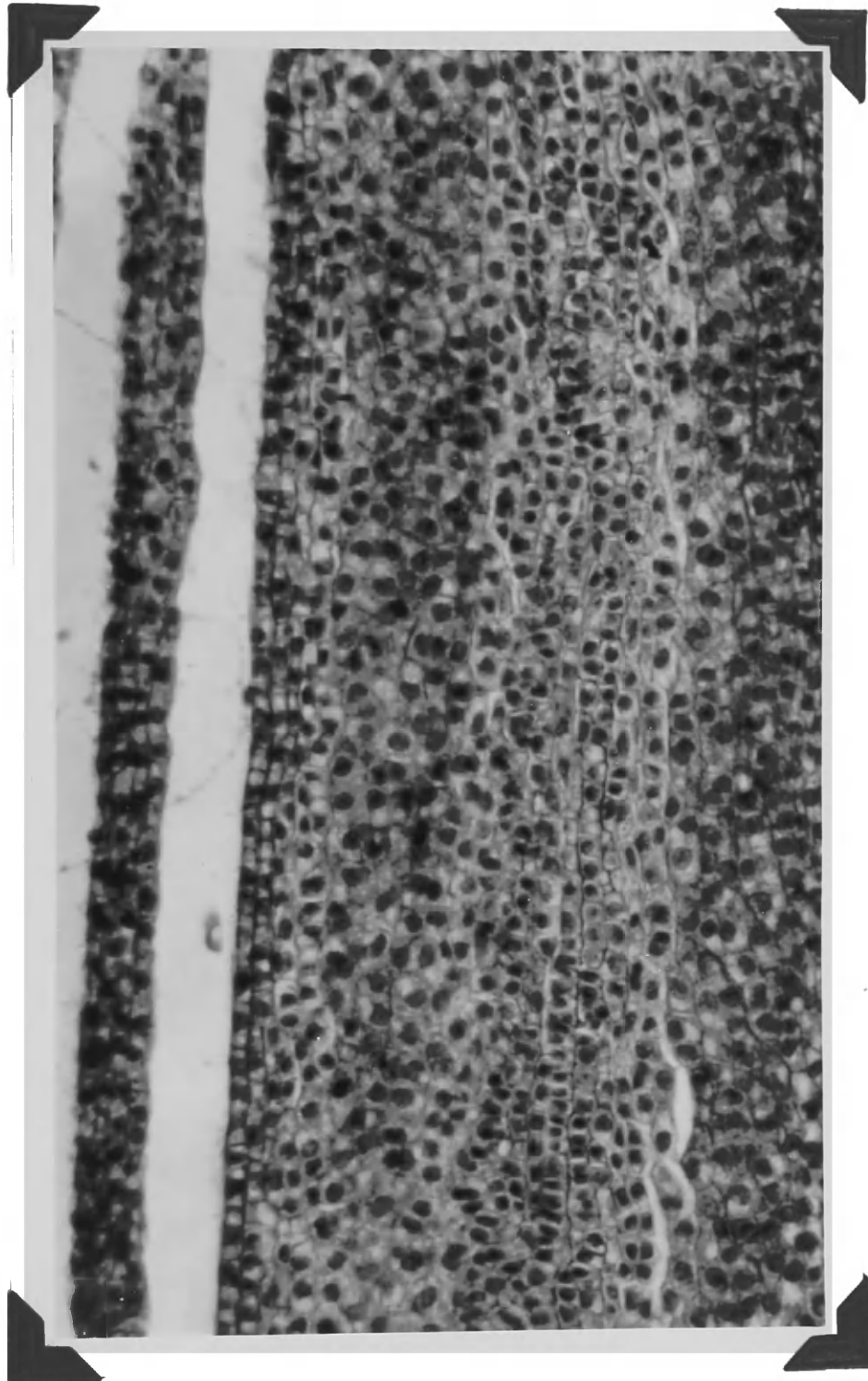


FIG.14

X300

Figure 15. 1933 Investigations

**Photomicrograph of a section showing the
sporogenous tissue in the anther of the flower
bud shown in Figure 9.**

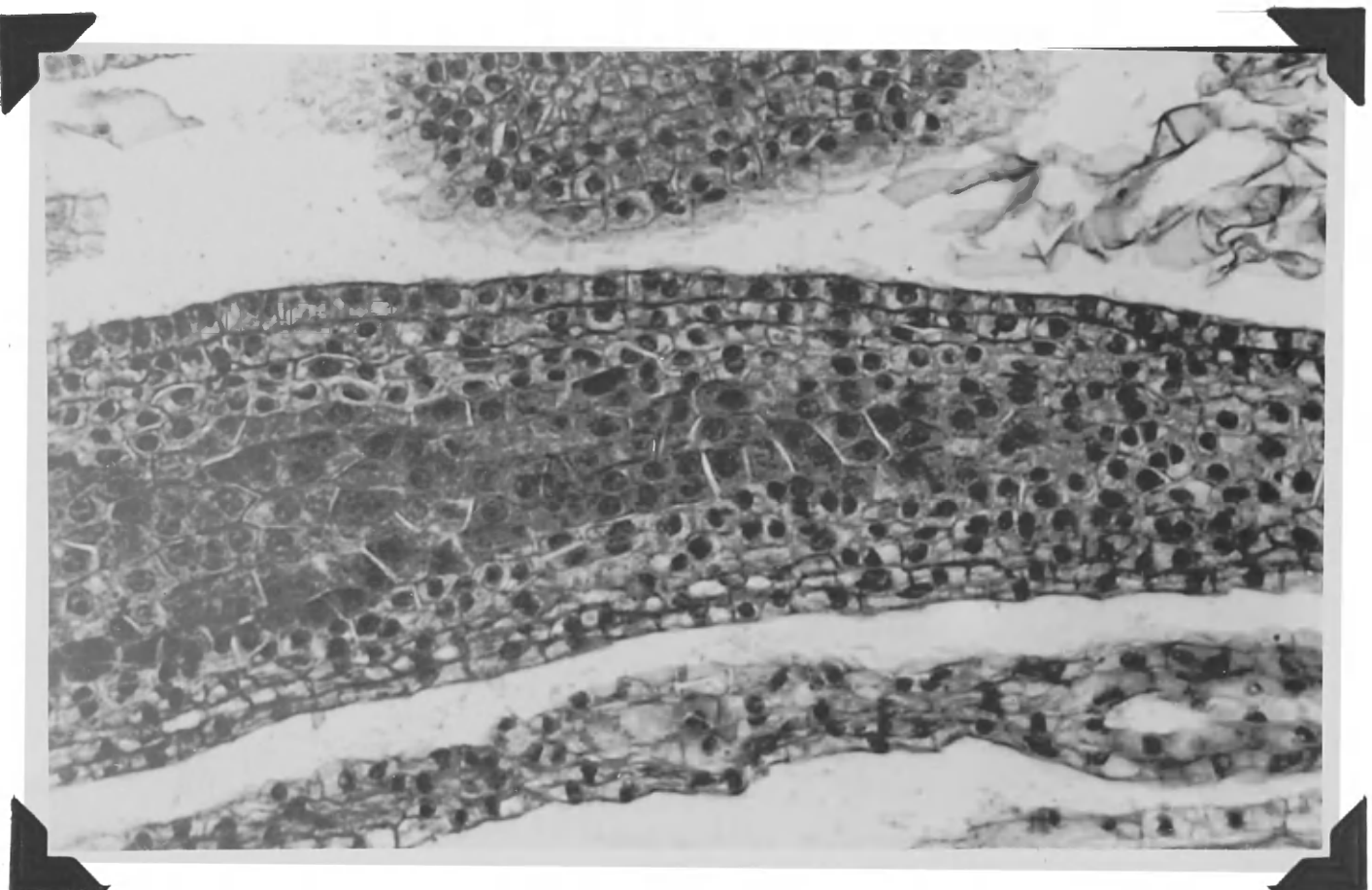


FIG. 15

x 300

Figure 16. 1933 Investigations

**Photomicrograph of a section showing young
pollen grains in the anther of the flower bud
shown in Figure 10.**



FIG. 16

X 300

Figure 17. 1933 Investigations

**Photomicrograph of a section showing the
breaking up of the tetrads in the anther of
the flower bud shown in Figure 11.**

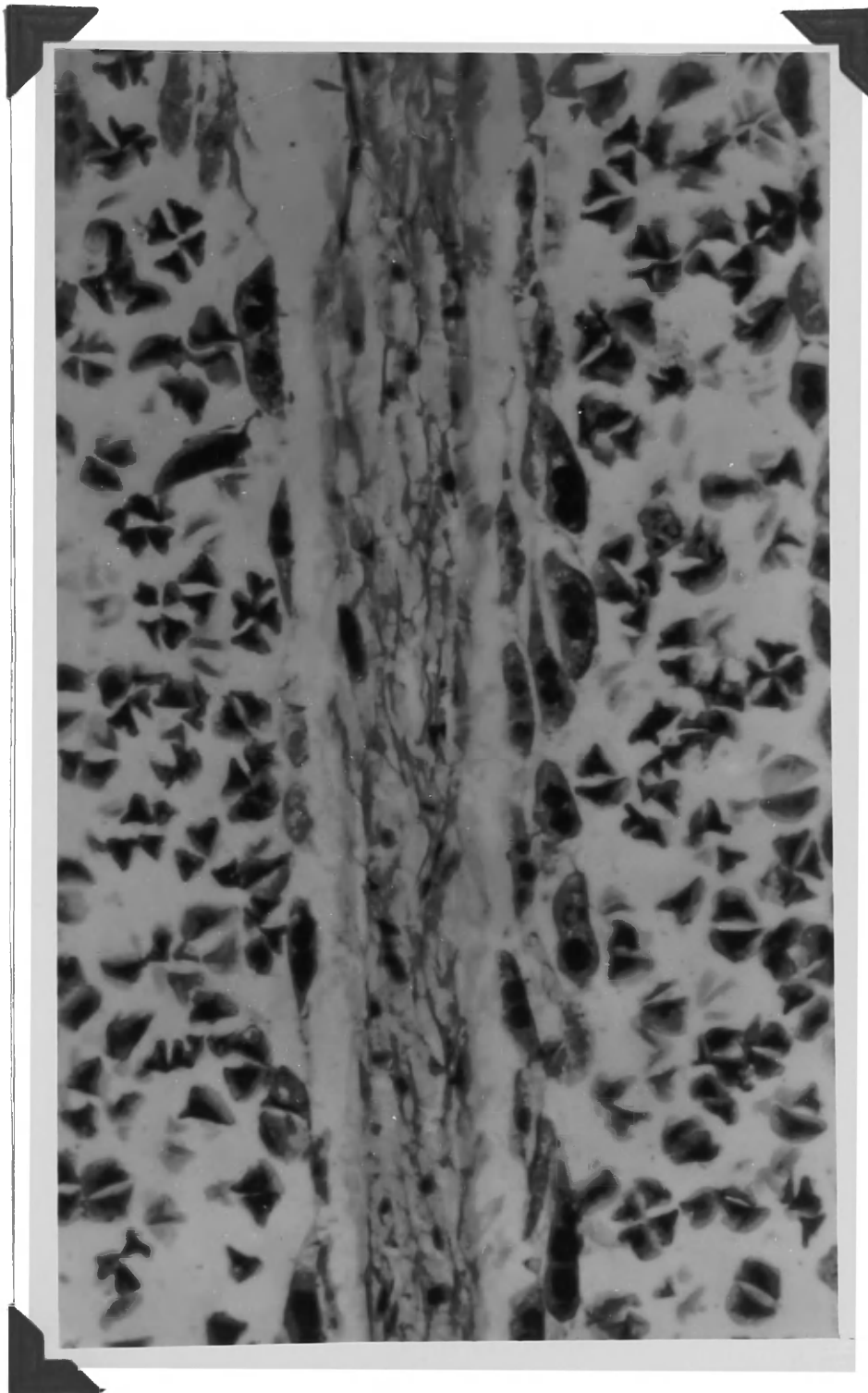


FIG.17

X300

Figure 18. 1933 Investigations

Photomicrograph of a section showing the
prophase of the first division of the pollen
mother cells in the anther of the flower bud
shown in Figure 12.

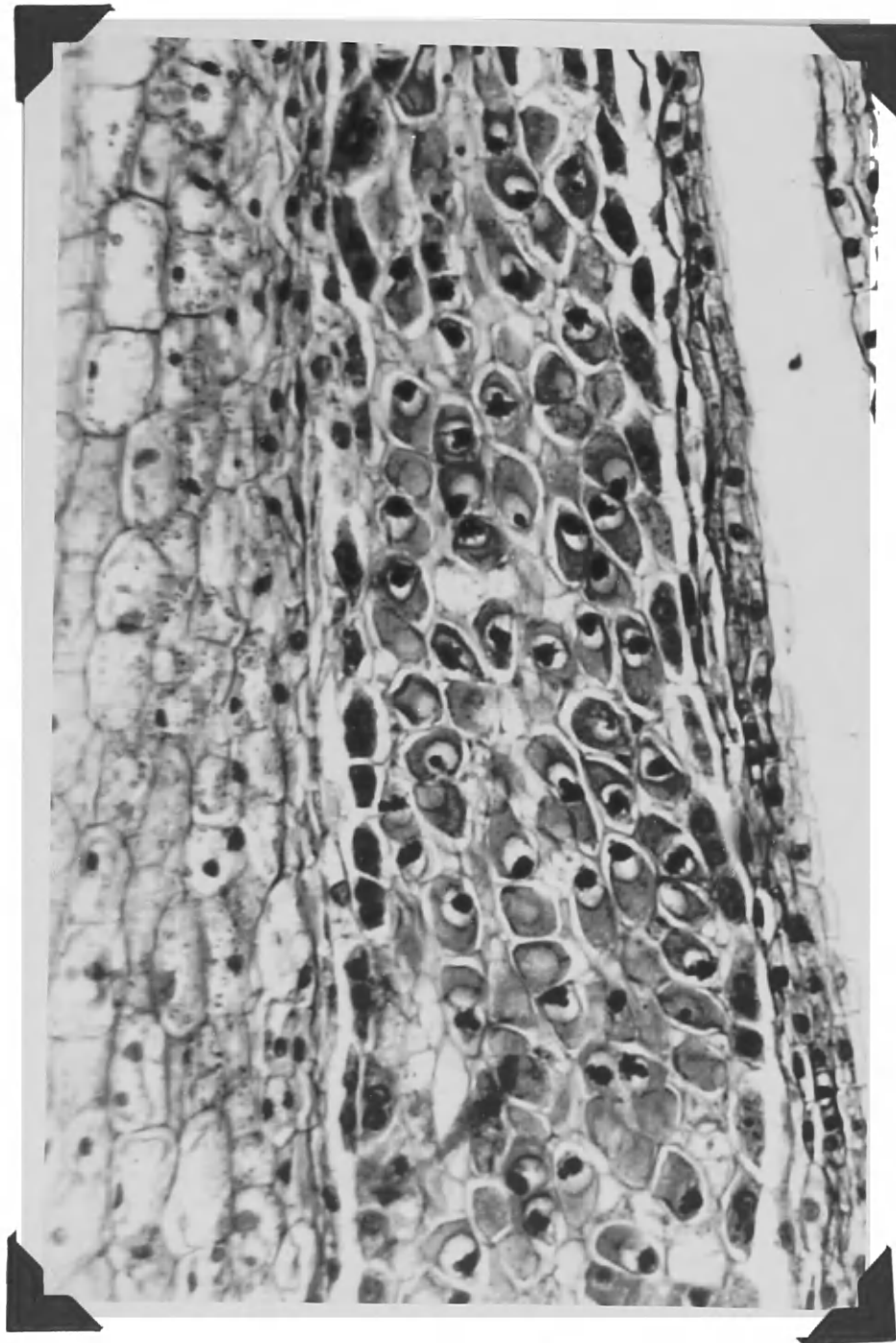


FIG. 18

X 300

Figures 19 and 20. 1933 Investigations

Photograph showing results on January 26,
1934, of forcing bulbs.

Figure 19 right "80° bulbs"

left "59° bulbs"

Figure 20 left "50° bulbs"

right "40° bulbs"

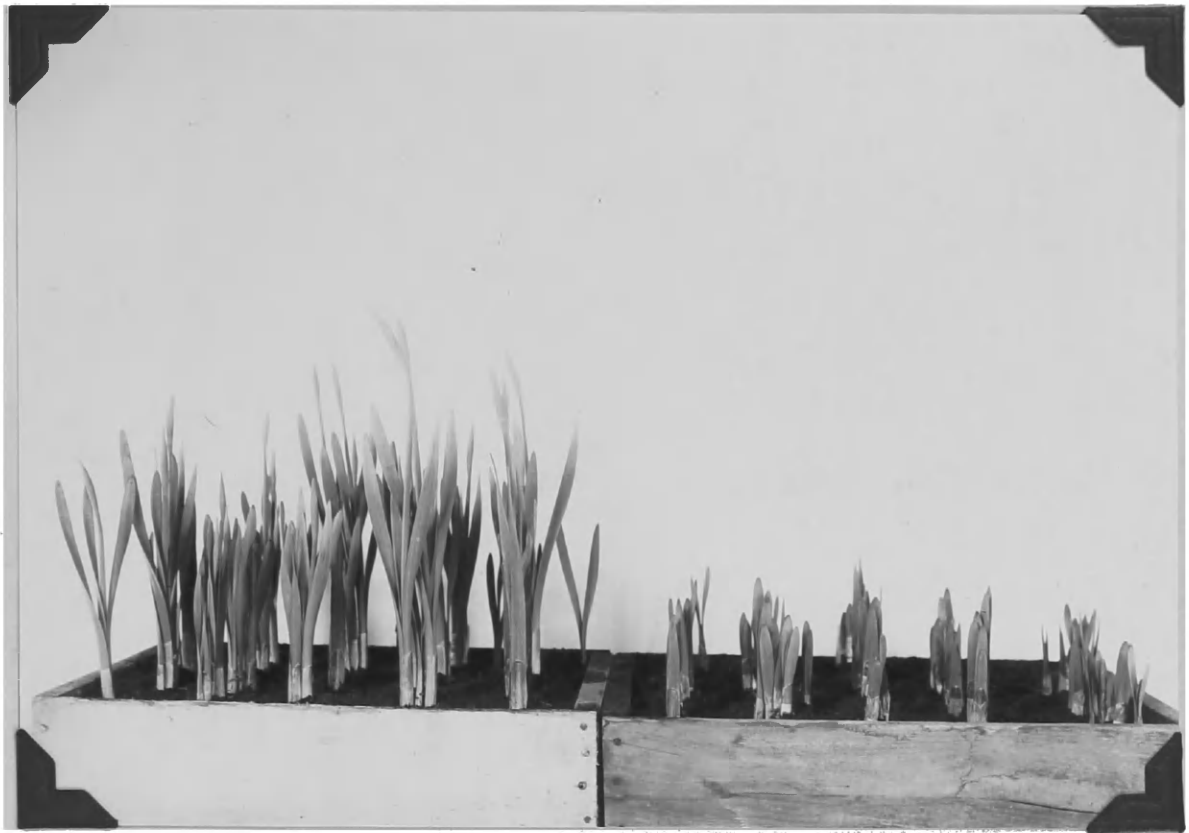


FIG. 19

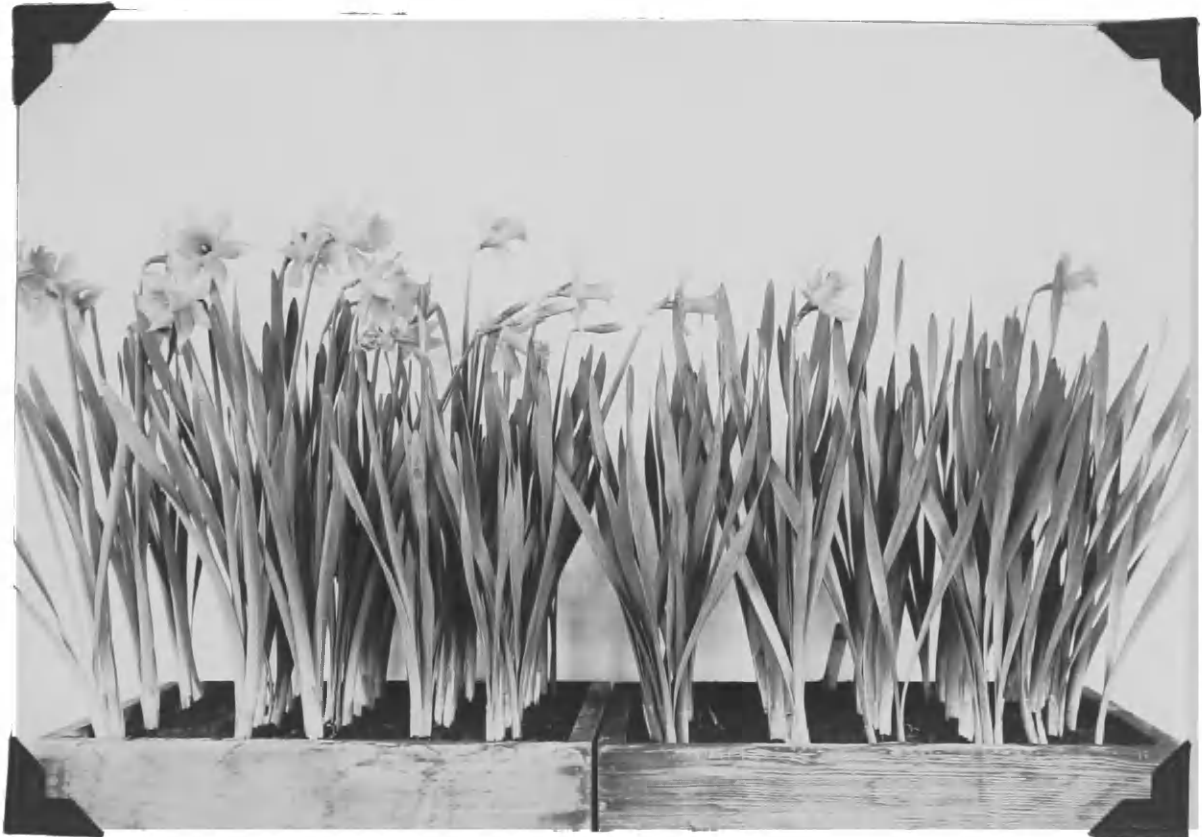


FIG. 20

Figure 21. 1933 Investigations

Photograph on March 5 of new vegetative buds at the bases of the scapes of four of the bulbs shown in Figures 19 and 20. Note the dead unelongated flower bud shown with the vegetative bud of the "40° bulb."

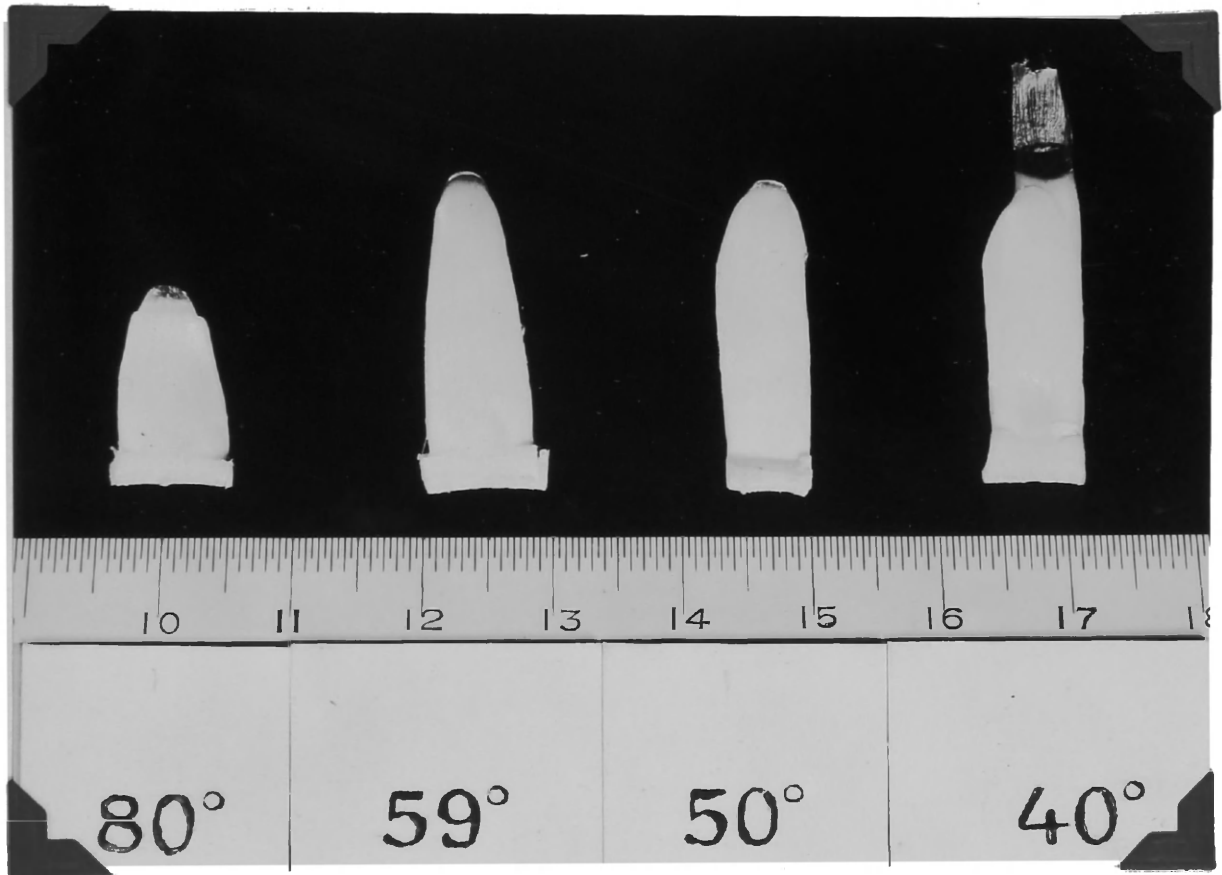


FIG. 21

LITERATURE CITED

1. American Daffodil Year Book
1935. Daffodil Literature, compiled by Alice C. Atwood.
American Horticultural Society.
2. Beijer, J. J., Slogteren, E. van
1932. Vroegbroei van Narcissen. Laboratorium voor
Bloembollenonderzoek te Lisse, Holland. August.
3. Blaauw, A. H., Hartsema, A. M., and Huismann, E.
1932. Temperatuur en Strekkingsperiode van de Narcis.
I. Proc. Kon. Akad. v. Wetensch. Amst. vol. 35, no. 6.
(Meded. No. 35 Labor. v. Plantenph. onderz., Wageningen,
Holland.)
4. Chamberlain, C. J.
1932. Methods in Plant Histology, 5th ed. University
of Chicago. 1901.
5. Denny, F. E.
1930. Sacrose and Starch Changes in Potatoes Treated with
Chemicals That Break the Rest Period. Am. Jour. Bot.
v. 17, pp 694-702. July.
6. Grainger, John
1935. Prednction of Early Daffodil Blooms. The Gardeners'
Chronicle. London. March 9.

7. Griffiths, David

1930. Daffodils. U. S. Dept. Agric. Circ. 122.

8. _____

1930. Notes on Daffodil Storage Tests in 1929. The Florists' Exchange. v. 74, no. 5, p. 48, Feb. 1, and no. 6, p. 15, Feb. 8.

9. _____

1930. Additional Notes on Storage of Daffodils in 1929. The Florists' Exchange. v. 73, no. 10, p. 13, Mar. 8.

10. _____

1930. Another Possible Method for Saving Our Daffodils. The Florists' Exchange. June 28.

11. _____

1935. The Magic 50° in Daffodil Forcing. Seed World. v. 37, no. 1. Jan. 4.

12. _____

1935. The Late Forcing of Daffodil Bulbs. Seed World. v. 37, no. 6. Mar. 15.

13. Griffiths, David; Wright, R. C.

1931. Daffodil Bicolor Victoria Blooms for Thanksgiving.
The Florists' Exchange. v. 76, no. 5. Jan. 31.

14. _____

1931. Late Forcing of Daffodils and Hyacinths. The
Florists' Exchange. v. 76, no. 15. Apr. 11.

15. _____

1932. Daffodil Forcing. The Florists' Exchange.
Apr. 16.

16. _____

1932. Effect of Storage Temperature on Date of
Flowering. Jour. Heredity. v. 23, no. 12. Nov.

17. Griffiths, David; Wright, R. C.; Lumsden, D. Victor

1931. Daffodil Storage and Forcing in 1930-31. The
Florists' Exchange. v. 76, no. 9, p. 9. Feb. 28.

18. Hasselbring, Heinrich

1927. Carbohydrate Transformations in Carrots
during Storage. Plant Physiology. v. 2, pp. 225-
243. July.

19. _____

1932. Effect of Hot-Water Treatment on the
Carbohydrate Changes in Narcissus Bulbs during
Storage. Plant Physiology. v. 7, pp. 145-154.

20. Hill, E. G.
1931. A Survey of the U. S. Bulb Industry. Bur. Agr.
Econ. United States Department of Agriculture. March.
21. Howard, W. L.
1915. An Experimental Study of the Rest Period in Plants.
The Summer Rest of Bulbs and Herbaceous Perennials, Second
Report. Mo. Agr. Expt. Sta. Research Bul. No. 15.
22. Huisman, Ebeline; Hartsema, Annie. M.
1933. De Periodieke Ontwikkeling van Narcissus pseudo-
narcissus L. Meded. Landb. hogeschool Wageningen. Dl. 37.
(Meded. No. 38 Labor. v. Plantenph. onders. Wageningen,
Holland.)
23. Lumsden, D. Victor
1932. Flower Development in Narcissus during Summer
Storage. Pro. Am. Soc. for Hort. Science. v. 29,
pp. 522-525.
24. Miller, E. C.
1931. Plant Physiology. 1st ed. McGraw-Hill Book Co.
25. Nagao, Seijin
Number and Behavior of Chromosomes in the Genus Narcissus.
Mem. Coll. of Sci. Kyoto Imp. Univ. Series B. v. 8,
no. 2, article 4.

26. Pinkhof, Marianne

1930. Untersuchungen Über die Umfallkrankheit der Tulpen. Laboratorium voor Bloembollenonderzoek te Lisse, Holland. No. 33. Jan.

27. Plant Quarantine and Control Administration.

1928-30. Report. United States Department of Agriculture.

28. Plant Quarantine and Control Administration.

1930. Service and Regulatory Announcement. United States Department of Agriculture.

29. Sachsse, R.

1877. Über die Stärkeformel und über Stärkebestimmungen. Chem. Centralb. 8: 732-736.

30. Slogteren, E. van

1923. Modern Methods of Combating Bulb Diseases. Rep. Internat. Conf. Phytopath. and Econ. Entomol. Holland. 150-162.

31. Slogteren, E. van

1933. The Early Forcing of Daffodils. The Daffodil Year Book, 1933. Royal Horticultural Society, London.

ACKNOWLEDGMENTS.

The writer wishes to acknowledge the cooperation and help of those who have been of assistance in carrying on these investigations.

Dr. J. H. Beaumont has given inestimable aid in the work, particularly in the presentation of the data. Dr. C. O. Appleman and Dr. Ronald Bamford have also been most helpful.

Dr. E. C. Auchter and Dr. V. R. Boswell have been of great service and have contributed much to the work. The late Dr. David Griffiths kindly supplied the bulbs used in these studies. Dr. E. V. Miller has rendered valuable service and advice during the sampling of the material and the running of the carbohydrate determinations. Mr. R. C. Wright is due credit for the cold storage facilities provided at Arlington Farm.

Approved,

J. H. Beaumont.

May 11, 1935