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PHYSIOLOGICAL EXPERIMENTS IN CONNECTION WITH FLOWER FORMATION IN WEDGWOOD IRIS (IRIS cv. "WEDGWOOD")

A. S. RODRIGUES PEREIRA

(Laboratory of Plant Physiological Research of the Agricultural University, Wageningen (Communic. No. 218), and Botanical Institute of the University of Nijmegen, Nijmegen)

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CHAPTER I

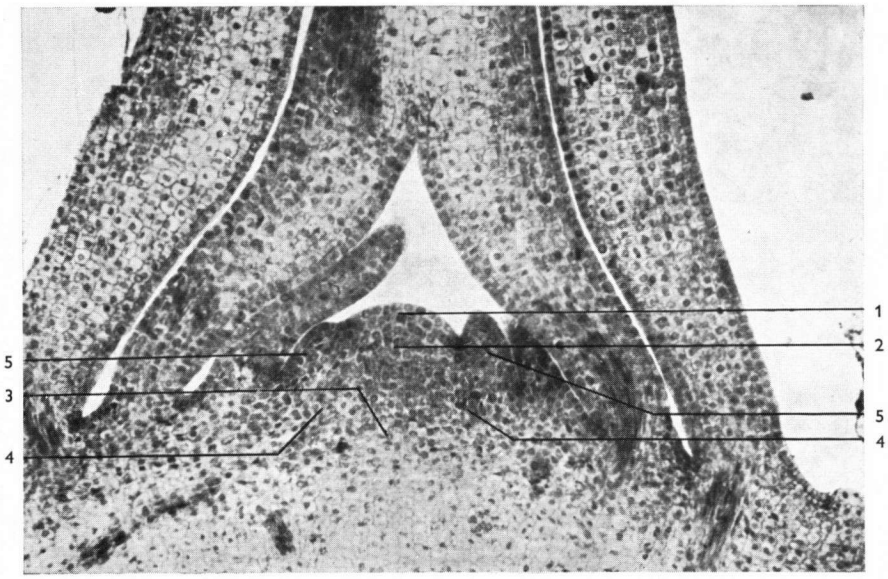
INTRODUCTION

In the early twenties BLAAUW started his investigations in connection with bulb growing with a morphological study on the life-cycle of the hyacinth, a study that was soon extended to other bulbous plants and to more physiological aspects. For many years these investigations were continued at the Laboratory for Plant Physiological Research at Wageningen. Research on bulbs is also carried out at the Laboratory for Bulb Research at Lisse.

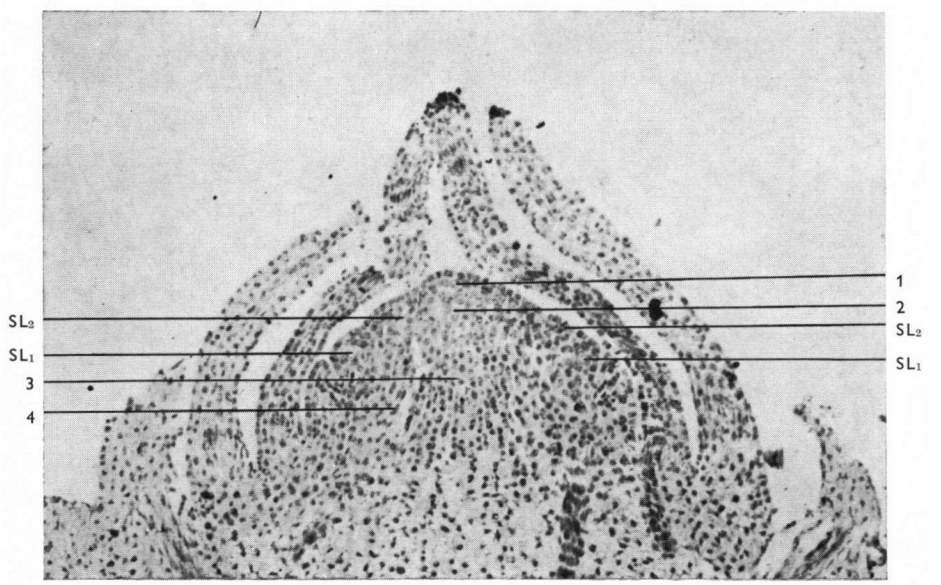
BLAAUW's first article on iris appeared in 1933; it was followed by a great number of papers both by himself and his collaborators, of whom Miss HARTSEMA is still continuing and extending his work. BEYER, at Lisse, particularly worked on the retarding of irises.

Originally, the studies by Blaauw and his group were carried out also in order to solve practical problems concerning the flowering of bulbous irises. It was found that, economically, better results could be obtained by applying certain narrowly defined temperature treatments to bulbs and plants. Gradually, however, more fundamental problems had to be dealt with. In particular, the influence of external conditions on flower initiation and on the development of the primordia was studied in greater detail.

Plate I. Photomicrographs of longisections of the shoot apex of Wedgwood iris.

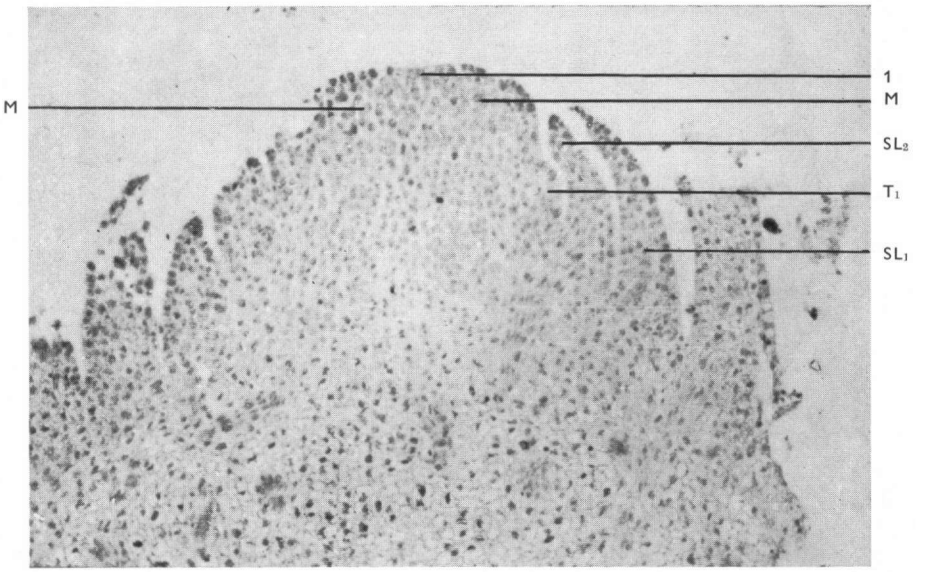


A. Vegetative shoot apex: (1), tunica; (2), central zone; (3), rib meristem; (4), provascular meristem; (5), young leaf primordia. × 64.

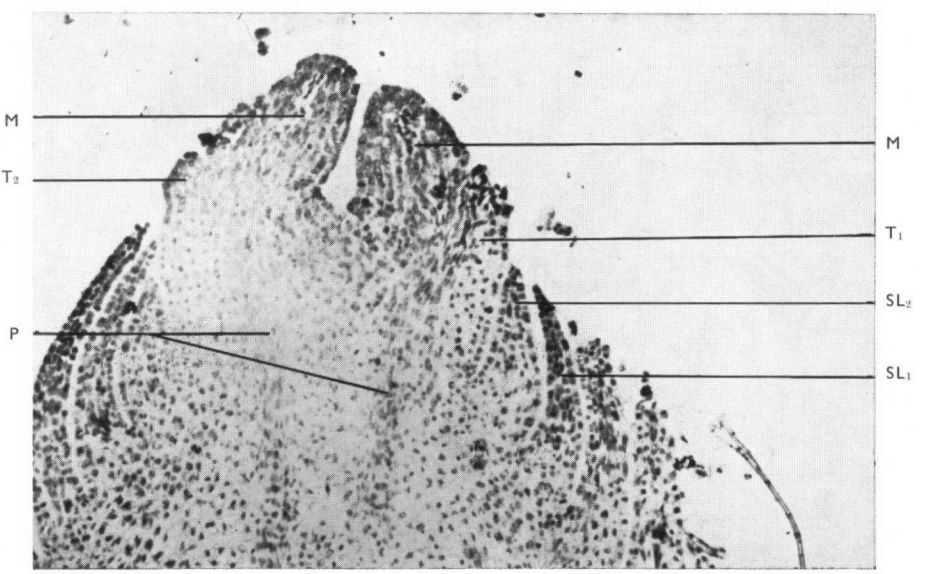


B. Transition into the reproductive stage, stage I–II: SL 1 and SL 2, first and second spatha leaf; (1), tunica; (2), central zone; (3), rib meristem; (4), provascular meristem. × 64.

Plate II. Photomicrographs of longisections of the shoot apex of Wedgwood iris.



A. Stage III: M, staminal primordium; T_1, inner tepal primordium. (1), tunica; SL_1 and SL_2, first and second spatha leaf. × 64.



B. Stage V: T_2, outer tepal primordium; P, provascular strand. SL_1 and SL_2, first and second spatha leaf; M, staminal primordium; T_1, inner tepal primordium. × 64.

Plate III. Photomicrographs of longisections of reproductive shoot apices of a bulb and an excised bud.

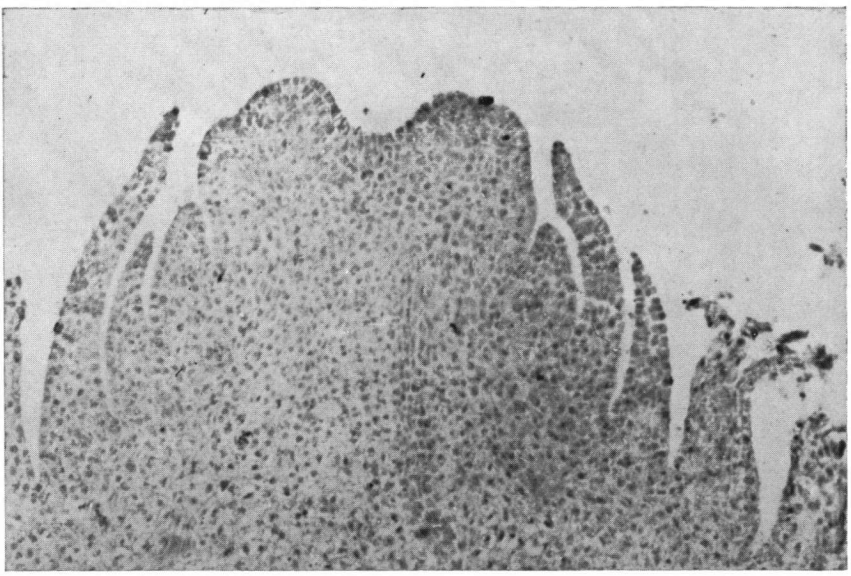


Fig. A. Shoot apex of a bulb; incubation 6 weeks at 13° C.—Stage III–IV. × 64.

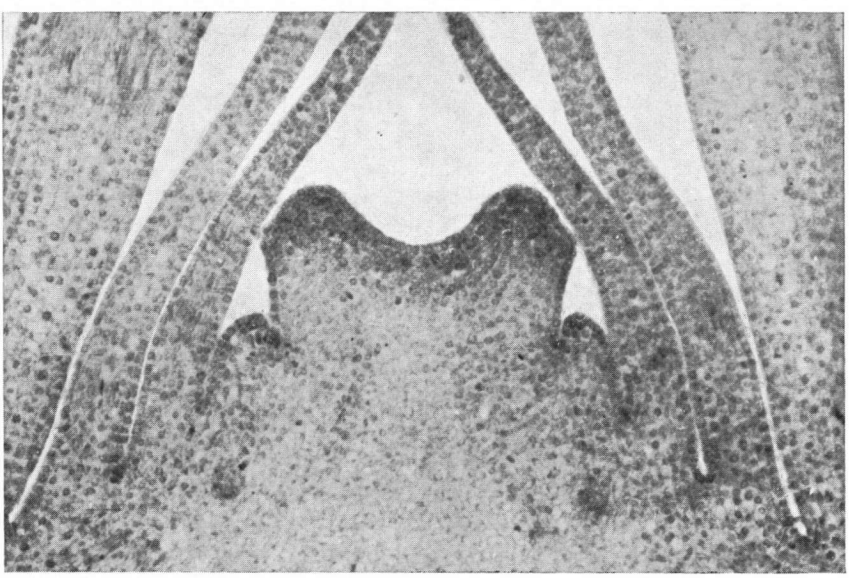


Fig. B. Shoot apex of an excised bud (b); incubation of the bulb before excision 4 weeks at 13° C, incubation of the bud after excision 2 weeks at 13° C. The shoot apex was still vegetative at the moment of excision. Stage III–IV. × 64.

The iris bulb is a very suitable object for experiments on the physiology of flowering: the bud is still vegetative at the time of digging, in the middle of August. The shoot apex rapidly proceeds to flower initiation under the influence of a cold treatment viz. at 13° C. This treatment may also be applied during dry storage.

In this study we are concerned with the problem of the influence of various factors on the transition of the shoot apex from the vegetative into the reproductive state in Wedgwood iris [1]) against the background of floral initiation in general. Particularly, we investigated whether the stimulus leading to floral initiation originates from the bud itself. To this purpose the developmental changes in isolated buds of Wedgwood iris, cultured on an agar medium under aseptic conditions, were studied, and the results compared with those obtained on explants consisting of the bud and part of the scales or of the already initiated leaf primordia. In all these cases the capacity of the buds to initiate flowers was tested. It appeared that both the scales and the leaves greatly influence flower formation.

It was investigated whether this is due to some specific flower inducing agent or to the unspecific action of carbohydrates in the scales. In this connection we studied the influence of various temperature treatments on the rate of translocation of the carbohydrates from the scales to the bud. In these experiments the effects of temperatures promoting flower initiation as well as of those retarding it were tested.

CHAPTER II

LITERATURE

1. Introduction

Blaauw never has presented a comprehensive review of the many investigations on flowering in bulbous plants, carried out by himself and his collaborators. He only presented a short treatise (1941) on the relation between flower formation and temperature in bulbous irises. Recently, however, Hartsema (1961) has reviewed bulb research as carried out during many years at Wageningen. In earlier years, a few reviews appeared, which discussed only a part or an aspect of bulb research in the Netherlands (Purvis, 1937 and 1938). Went (1948,1953) discussed part of the earlier work by Blaauw and his group in his articles on themoperiodicity. In later years, a number of reviews on the physiology of flowering appeared of which only those by Naylor (1953), Liverman (1955), Doorenbos & Wellensiek (1959) and the annual chapters by Lang in "Fortschritte der Botanik" may be mentioned here, as they are of interest for the problem discussed in this paper.

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[1]) Wedgwood iris has been developed as a cultivar of the "Hollandse iris". The latter has been breeded from crosses between *I. Xiphium praecox*, *I. tingitana* and *I. lusitanica*.

2. Discussion of some theories concerning the changes in the morphological structure of the shoot apex during flower initiation

In this section the changes in the morphological structure occurring during the development of the flower primordium, as given by different authors, will be discussed.

According to Buder & Schmidt, the morphological structure of the vegetative shoot apex may be described in terms of planes of cell division (A. Schmidt, 1924). The outer cell-layers (up to three) differ from the underlying tissue by their anticlinal cell divisions. This part is called the tunica. The tissue enclosed by the tunica is called the corpus, the cells of which divide along various planes.

By differential staining methods a cytohistological zonation pattern becomes visible which does not coincide with the tunica-corpus pattern. The zonation seems to reflect the integrated functions of the cells by virtue of their position.

Apart from the way in which the situation in the vegetative shoot apex is described, two opposing views are presented regarding the changes occurring at the transition into the reproductive stage.

Grégoire (1938) stressed the general observation that a flower structurally and typologically differs from the leafy shoot. In the floral apex a peripherous meristem and a parenchymatous core can be observed, but within the meristematic layer Grégoire could not distinguish between a tunica and a corpus. Therefore, in his opinion the floral apex is not a modification of the vegetative shoot apex, although it originates from the latter; he considered it rather as a lateral outgrowth, constituting a formation *sui generis*.

Grégoire fully realized that one could only come to a decision on this point if one would be able to trace the earliest origins of the floral receptacle, but in this respect his investigations remained incomplete.

According to his opponents, more emphasis should be laid on the fact that the reproductive apex develops organically from the vegetative one. Philipson (1947, 1949) showed that the zonation pattern of *Succisa pratensis* gradually vanishes during transformation into the reproductive phase. According to this author the meristematous mantle consists of the tunica and the outer corpus layers which parts can also be distinguished in the reproductive apex.

In the course of their life-cycle studies on apical meristems, Rauh & Reznik (1951, 1953) underlined the histogenetic significance of the cytohistological zonation pattern. On account of these investigations they, as well as Troll & Weber (1954), rejected Grégoire's views. Gifford (1954), holding the same opinion, pointed out that the emphasis laid upon the histogenetic significance of the various zones, e.g. by Rauh & Reznik, must be seriously questioned.

Buvat (1951 a and b, 1952) made an attempt to reconcile the conflicting ideas. In the vegetative apex itself, only a small number of mitotic figures are observed (Lance, 1952, 1953). The cells generally seem to be in a state of mitotic inactivity, constituting a

so-called "méristème d'attente". They only become active when a terminal flower or inflorescence is to be formed. CLOWES (1959) pointed out that the frequency of mitotic figures as used by Buvat and Lance, is a poor indication of meristematic activity, since the period during which the nucleus is in mitosis is short and moreover variable, in relation to the time spent in interphase.

Preliminary histochemical work by WETMORE *et al.* (1959) seems to indicate greater amounts of oxidases and dehydrogenases in the mitochondria of the cells of the central zone over those in the peripheral or rib meristem zones.

These results find support in the detailed investigations by SUNDERLAND, HEYES AND BROWN (1957) who, through the use of minute Cartesian divers, found that the respiration rate in the large-celled core of the apical dome is much higher than in the small-celled tunica.

On the other hand SALISBURY (1961) rightly points out that Buvat's theory, if it were true, would implicate that only the cells of the "méristème d'attente" respond to flowering hormone. From the investigations of WETMORE *et al.* (1959), however, it seems that starting from the central zone the entire apical meristem ultimately becomes involved in the formation of the flower primordium.

3. THE INFLUENCE OF TEMPERATURE ON THE TRANSITION FROM THE VEGETATIVE PHASE INTO THE REPRODUCTIVE ONE IN IRIS

BLAAUW (1934) indicated that a minimum size of the bulbs and a definite temperature treatment are essential for flower formation.

When, after lifting (at approximately 18° C) the bulbs are stored at temperatures around 25.5° C, flower inducing activities are strongly inhibited; the bulbs remain in a vegetative state. Therefore, 25.5° C has generally been accepted as the most favourable storage temperature, both in practice and in the laboratory (BEYER, 1952; HARTSEMA & LUYTEN, 1940, 1955).

At a temperature between 20° and 25.5° C, flower primordia are formed, which soon abort.

When, however, after lifting, the bulbs are kept at temperatures between 2° and 20° C, flower formation may follow. The rate of flower formation depends on the storage temperature. For Wedgwood iris the optimum temperature is 13° C at which floral induction occurs in 85 per cent of the bulbs (HARTSEMA & LUYTEN, 1955).

The rate of flower formation increases when, previous to storage at 13° C, the bulbs are kept at a temperature above 20° C for 1 to 5 weeks; in that case all the bulbs form flowers. The higher the temperature, the shorter the time required. The highest temperature applied was 33° C during one week (BLAAUW, LUYTEN & HARTSEMA, 1936; HARTSEMA & LUYTEN, 1940 and 1955). On the other hand a low temperature treatment (2° to 9° C) during 5 to 10 weeks decreases the chance on flower formation. The lower the temperature, the stronger the after-effect. As may be expected, this effect coun-

teracts the favourable effect of the high temperature treatment (BLAAUW, 1941).

4. FLOWER INITIATION IN IRIS, AND VERNALIZATION

It was mentioned in the preceding section that flower formation is most promoted by keeping the bulbs at temperatures between 9° and 15° C for a prolonged period. We will discuss whether it is justified to identify this phenomenon with vernalization. Vernalization is a temperature treatment, shortening the vegetative period. According to WELLENSIEK (1956) originally the Russian term "jarovizatzia" was used in any instance in which the vegetative period was reduced by the action of an environmental factor. The use of the word vernalization, being the translation of "jarovizatzia" is now restricted to those cases in which flower initiation is accelerated by a cold treatment. WELLENSIEK, DOORENBOS and ZEEVAART (1955) emphasize that the actual process of flower formation does not take place during the low temperature treatment, but afterwards, at another temperature.

The bulbous irises are mediterranean in origin and are steppe plants. In order to stimulate early flowering at our latitude, a forcing low temperature treatment is applied. In their natural environment temperature might be supposed to be low only during the period of flower formation. In fact it may be inferred from our own observations that bulbs having been stored at 25.5° C for over six months, may finally proceed to flower formation.

When the bulbs had been incubated at 13° C for a short period, the process of flower formation during a subsequent treatment at 25.5° C was accelerated. This might be looked upon as a vernalization process. Nevertheless, as flower formation occurs in bulbs kept at 13° C, the use of the term "vernalization" is not justified.

One arrives at the same conclusion by considering that it is necessary to continue the 13° C treatment of the iris bulbs until the shoot apex has become reproductive. In the case of genuine vernalization, the shoot apex is never reproductive at the end of the cold treatment.

5. INTERNAL FACTORS INFLUENCING FLORAL INITIATION

Internal factors, active in flower formation, can be studied by means of grafting experiments. The results of many of these experiments strongly suggest the existence of flower forming substances, and have contributed considerably to our knowledge concerning their nature. Arbitrarily the investigations by ZEEVAART (1957) may be quoted. He grafted single donor leaves from a short-day induced plant of *Perilla crispa* onto long-day receptor stocks, remaining in long days. After about three weeks the leaves were removed and immediately regrafted onto another group of long-day stocks. This procedure was repeated once more. It appeared that the donor leaves were still able to induce flowering after the third regrafting, 64 days after the last inductive cycle.

A relationship between nucleic acid metabolism and the process of flower induction has first been suggested by LANG (1955). KONAREV (1954) described an experiment in which an increase in ribonucleic acid (RNA)-phosphor was observed after vernalization of certain rye varieties; no increase could be demonstrated in unvernalizable varieties. It is open to discussion, however, whether this difference resulted from the vernalization, as it may be that during the cold treatment the development had already started. FINCH & CARR (1956), limiting the moisture content of Petkus rye during vernalization to 50 per cent of the dry weight, did not observe any changes in the content of RNA-phosphor, deoxyribonucleic acid (DNA)-phosphor and DNA-nitrogen.

KESSLER, BAK & COHEN (1959) sprayed fruit trees with solutions of purines and pyrimidines and in some cases obtained significant increases in the number of flowers. The increase in flowering was parallelled by an increased protein-N content and RNA/DNA ratio. The results are somewhat contradictory, however, and, since spraying was carried out on already induced branches, it does not seem possible to attach decisive value to these experiments. SALISBURY & BONNER (1960) dipped leaves and buds of *Xanthium*-plants in solutions of 5-fluorouracil and saw an inhibition of the formation of the flower primordia. The inhibition was reversed by orotic acid but not by uracil and thymidine. It only occurred when 5-fluorouracil was applied during the inductive dark period. From these experiments it seems clear that some process, connected with nucleic acid metabolism and essential to the realization of the induction, takes place in the bud in the time between pigment conversion and the translocation of the flowering hormone or its precursor to the bud. Investigations by HESS (1959, 1961) and CHAILAKHIAN *et al.* (1961) point into the same direction.

Many attempts have been made to isolate the flower forming substances. Those by MELCHERS & LANG (1941), PURVIS & GREGORY (1953) and NAPP-ZINN (1956) gave no positive result. ROBERTS (1951) obtained a crude extract from a flowering *Xanthium* which was reported to induce flower formation. HIGHKIN (1955) prepared a diffusate from peas, germinating under vernalizing conditions at 4° C. The diffusate significantly accelerated flower formation in non-cold-treated, but vernalizable pea varieties; it did not contain auxin in measurable quantities.

SIRONVAL (1950, 1957) sprayed young strawberry plants with an unsaponifiable petroleum ether fraction of the leaves dissolved in ether. He obtained an increase of 50 – 100% in the number of flowers, depending on the development of his testplants. The fraction contained vitamin E which, in minute quantities, proved to have a marked flowering promoting effect: about 2 μg per plant gave a 50% increase in flowering. A second fraction, containing sterols, had a stimulating effect too, although to a smaller degree: 60 mg per 24 plants gave a 50% increase in flowering. BOUILLENNE (1955) suggested that products of the hydrolytic activity of chlorophyllase,

e.g. the vitamins E and K, might be components of florigen. From the experiments of SIRONVAL (1957) it might be concluded that an increased hydrolytic activity of chlorophyllase occurs during the transition from the vegetative into the reproductive stage.

Other chemically well defined compounds which might have a flowering promoting action are auxins and gibberellins. Gibberellic acid promotes flower formation in long-day plants but not in short-day plants. CURRY & WASSINK (1956) found that application of gibberellic acid to *Hyoscyamus niger* (annual strain) grown under long-day conditions in narrowly defined spectral regions of green and red, which are reported to inhibit both bolting and flower formation (STOLWIJK & ZEEVAART, 1955), resulted in stem elongation and flower bud formation. They also found that stem elongation and flower bud formation were always closely associated. The same was observed by CHAILAKHIAN (1958) for a great many long-day varieties. He found that in short-day plants stem elongation is also promoted by gibberellic acid but no flowers are induced. He inferred that the complex of flowering hormones, or florigen, consists of two groups of compounds, viz. gibberellins for stem formation or elongation and so-called anthesins for flower formation. The phenomenon that long-day plants do not form flowers under short-day conditions may be due to a shortage of gibberellins; the absence of anthesins might be responsable for the non-flowering of short-day plants under longday conditions. This theory accounts for many phenomena observed in short-day and long-day plants.

In experiments of LANG, SANDOVAL & BEDRI (1957) application of endosperm of *Echinocystis macrocarpa* (Cucurbitaceae) caused bolting and flowering in biennial *Hyoscyamus niger* and in the long-day plant *Samolus parviflorus* held under non-inductive conditions. In PHINNEY's dwarf maize test (1956) this material had been shown to contain substances which behave exactly like gibberellins.

HARADA (1960) isolated gibberellin-like compounds from the long-day plant *Rudbeckia speciosa* and the day-neutral *Chrysanthemum* variety "Shuokan". The substance extracted from induced *Rudbeckia* plants was able to induce flowering in *Rudbeckia*'s grown in short days. The substance extracted from vernalized "Shuokan" chrysanthemums induced flowering in non-vernalized plants of this variety (HARADA & NITSCH (1959a)). The authors worked with a highly purified, cristalline preparation. CHAILAKHIAN (1959) claimes to have made extracts from leaves of "Mammoth" tobacco and *Perilla nankinensis* that induced flowering in his *Rudbeckia bicolor* test plants, held under non-inductive conditions.

6. CONCLUSION

In recent years, various theories have been put forward to elucidate the phenomena observed at the transition of the vegetative state into the reproductive state. It is assumed that specific compounds

are formed, the flowering hormones, which will change the metabolism of the growing point. Except the promising experiments of Sironval and of Harada and Nitsch attempts to isolate and purify these compounds have not yet been successful. At least in some instances the characteristics of the active substances point into the direction of the gibberellins.

CHAPTER III

MATERIAL AND METHODS. PRELIMINARY EXPERIMENTS

1. Material

In the second half of August, Wedgwood iris bulbs, having an average circumference of 10½ to 11 cm and a fresh weight of 22 to 26 g, were obtained from a nursery. They were stored at 25.5° C.

2. Methods

2.1. *General*

The stage of development of a growing point was determined by observation of the isolated apex with a binocular microscope (enlargement 40 ×).

Duplicate determinations of the amount of dry material, the water content and the amount of various carbohydrates were carried out on samples of 15 bulbs. Scales and stem-discs (bulbs from which the scales had been removed) were analyzed separately. From the scales a sample of 12 – 15 g fresh weight was taken by longitudinal cuts. The buds were divided into four parts, one quarter of a bud was analyzed; the total fresh weight of all the quarters was 12–15 g. The ratio of the fresh weight of each sample to the total fresh weight of the material was known. The results are recorded per bulb as an average of the bulbs used for the respiration measurements.

All measurements were carried out in duplicate.

2.2. *Respiration*

The respiration rate was measured both on whole bulbs and buds. The fresh weight of the samples was determined.

Carbon dioxide production and oxygen uptake of five intact bulbs (or five buds) at a time were measured with a diaferometer according to Noyons (1922, 1937). For a detailed description of the apparatus employed reference may be made to Spierings, Harris & Wassink (1952). In the experiments with intact bulbs, the container had a content of about 200 ml; the container of the buds had a content of 15 – 100 ml, depending on the size of the buds. Air saturated with water at the temperature of the container passed through at a rate of 4 litres per hour. Unless indicated otherwise, respiration rates are expressed in milliliters of oxygen per item per hour. Carbon

dioxide and oxygen measurements on the same sample, carried out with an interval of about 25 minutes agreed to within 3% and 2% respectively (average of 6 experiments).

2.3. *Dry weight*

The material was cut into small pieces of about ⅛-½ ml and dried in flat tins in a ventilated oven at 70° C for about 20 hours. Afterwards it was transferred into drying flasks and heated at 105° C to constant weight. The standard deviation of the dry weight measurements amounted to 5.7 % of the value obtained.

2.4. *Carbohydrates*

2.4.1. Soluble corbohydrates

The samples, of 5-15 g fresh weight, were cut into small pieces, weighed and killed in 35 ml boiling ethanol (96 %). After cooling the material was ground, extracted for half an hour with 70 % ethanol, and decanted. This procedure was repeated twice. The extracts were put together and the ethanol removed in vacuo at 45° C.

Clarification was achieved by adding a solution of neutral lead acetate; the excess of lead was removed with di-sodium phosphate. The extract was neutralized, afterwards diluted 40 times, and brought to a known volume. In this solution the reducing sugars and laevulose were determined according to V. D. PLANK (1936). Another part of the extract was hydrolyzed, diluted 300 times and submitted to the same determination procedure. From the values obtained, the contents of dextrose, laevulose, sucrose and polyfructosides were calculated. Duplicate determinations agreed to within 5 %. The standard deviation of the carbohydrate measurements amounted to 7 % of the value obtained.

It appeared that the soluble carbohydrates were almost quantitatively extracted by the procedure described. The combined fourth, fifth and sixth extracts contained only negligeable amounts of sugars.

Iris bulbs contain large amounts of polyfructosides. SCHLUBACH *et al.* (1933, 1934) made a special study of them in the rhizomes of irises and isolated irisin.

In the non-hydrolyzed extract laevulose appeared to be absent.

2.4.2. Insoluble carbohydrates

Starch determinations according to PUCHER, LEAVENWORTH and VICKERY (1948) were carried out on 100 mg of powdered dry material. The dilution factor at titration was approximately 50.

3. PRELIMINARY RESPIRATION MEASUREMENTS

The respiration rate of the bulbs stored at 25.5° C was measured (Exp. 1). Immediately after lifting, it was found to decrease rapidly; after two weeks the rate was about half of the original value. After this period the decline was only small (Fig. 1).

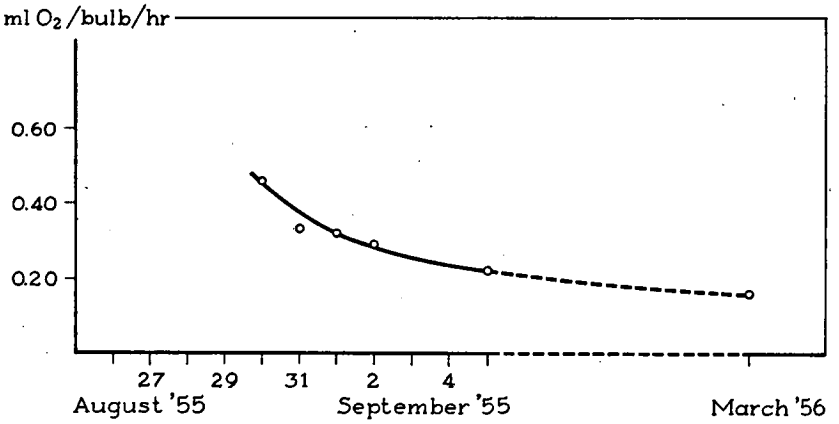

Fig. 1. Respiration rate of Wedgwood iris bulbs during storage (Exp. 1). 1955/1956 —Each sample contained 5 bulbs, total weight approximately 100 g. Storage temperature 25.5° C.

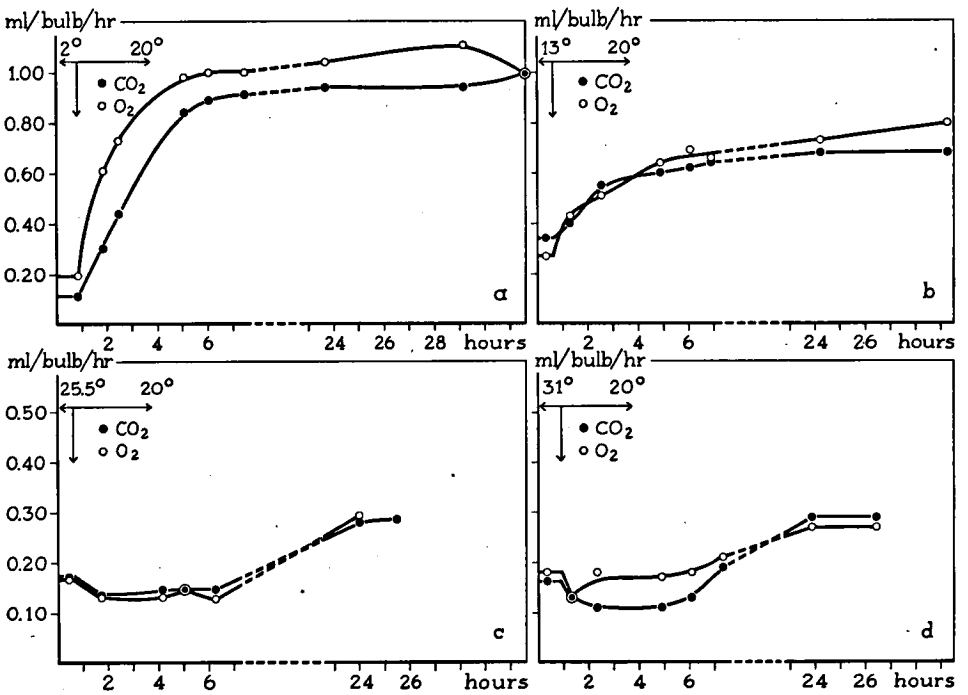

Fig. 2. Influence of a sudden change in storage temperature on the respiration rate of Wedgwood iris bulbs (Exp. 2). Storage temperature of all the bulbs 25.5° C until March, 10, 1956. At this date the bulbs of Exp. a were stored at 2° C, those of Exp. b at 13° C and of Exp. d at 31° C, whereas those of Exp. c remained at 25.5° C. On March 25 (↓) all the bulbs were transferred to 20° C.—Other data as in Fig. 1.

The respiration intensity varied largely in the different seasons, but a decline as shown in Fig. 1 was always observed. The experiments described in the following sections were carried out after the rapid decline in respiration rate had passed.

Next, the influence of a sudden change in temperature on the respiration rate was determined (Exp. 2). In March 1956 the bulbs were stored at 31°, 25.5°, 13°, and 2° C respectively. After a few weeks, the respiration rates at these temperatures were determined and appeared to be constant. Immediately afterwards the temperature of the containers was shifted to 20° C; the measurements were continued for another forty hours (Fig. 2).

A thermo-couple was inserted into the interior of the bulb in order to determine the time required to reach temperature equilibrium. In the case of a transfer from 13° to 20° C or from 25.5° to 20° C about one hour was needed; transfer from 31° to 20° C required 1½ hour and from 2° to 20° C about two hours to abolish the temperature difference.

When the storage temperature suddenly rose from 2° or 13° to 20° C, the respiration rate increased (Fig. 2; a, b). This increase continued for some time after the interior of the bulb had adopted the new temperature; a constant value was obtained after about 24 hours.

When the temperature of the environment decreased from 31° or 25.5° to 20° C the respiration rate suddenly decreased (Fig. 2; c, d); the Q_{10} being about 1.4. After a few hours a minimum was reached, and a rise set in. Twenty-four hours after the fall in temperature, the respiration rate had attained a new constant level fifty to hundred per cent above the original one. It appeared that the bulb, in spite of the drop in temperature, eventually established itself on a higher level of activity. This confirms the results discussed in Chapter II from which it appeared that the metabolism of iris bulbs is inhibited at temperatures above 20° C. In bulbs stored at 20° C the respiration rate may rise further because normal developmental processes start.

In many experiments the dry outer scales had to be removed. Consequently it was of interest to investigate whether the respiration rate was affected by the removal of these scales (Exp. 3, Fig. 3).

After the removal of the dry scales, the respiration rate rapidly increased within 24 hours. This increase is partly due to the change in temperature (Exp. 2). A maximum was reached between 24 and 72 hours, it was followed by a decrease. Four days later the respiration rate was still about twice as high as at the time of the removal of the dry scales. On visual examination, no indications of bacterial or fungal infection could be observed.

It does not seem likely that the initial rise was due to a wound effect since all possible care was taken not to cut the subjacent fleshy scales. Moreover, only small notches being made in the dry scales without damaging the underlying tissue, the respiration rate and especially the carbon dioxide production suddenly increased. For

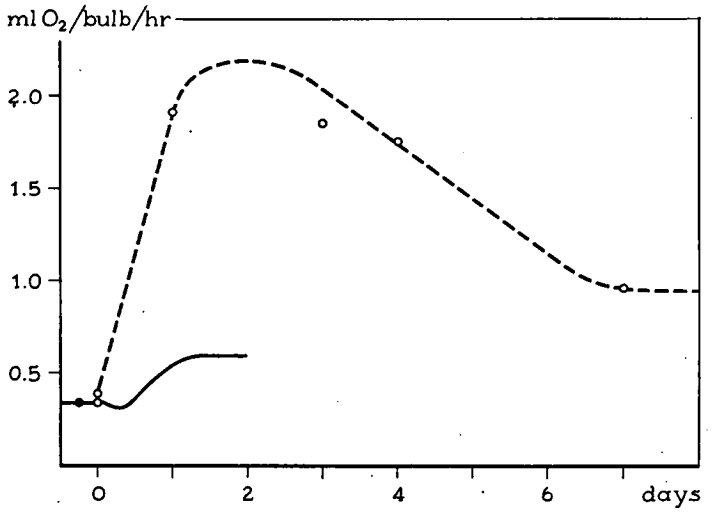

Fig. 3. The influence of the removal of the dry outer scales on the respiration rate of Wedgwood iris bulbs (Exp. 3). September 1955.—At $t = 0$ bulbs were transferred from 25.5° to 20° C, and the dry outer scales removed.—Circles: bulbs without dry scales; solid line: normal bulbs.—Other data as in Fig. 1.

that reason one may assume that the increase in respiration rate following the removal of the dry scales is due to the removal of an oxygen deficiency of the bulb tissue.

In order to test this assumption, the air in the container was replaced by oxygen and the carbon dioxide production measured (Exp. 4).

Exp. 4. The carbon dioxide production of intact bulbs in air and in pure oxygen. March 1956.—Bulbs with dry scales. In air: 15 bulbs; in oxygen: 10 bulbs.—Storage temperature 25.5° C; 45 minutes before $t = 0$ the bulbs were shifted from 25.5° to 20° C.—Other data as in Fig. 1.

Exp.	Time	Composition of the gas mixture in the container	
		20 % O_2 80 % N_2	100 % O_2 0 % N_2
		ml CO_2 / bulb / hr	ml CO_2 / bulb / hr
I	$t = 0$	0.14	
	$t = 18$ hours	0.28	
II	$t = 0$	0.18	
	$t = 20$ minutes		0.22
	$t = 18$ hours		0.35

The respiration rate of both samples, in air and in pure oxygen, increased by a factor 2 within 18 hours after transfer of the bulbs from 25.5° to 28° C. Comparing the initial values in Exps. I and II, we feel that there is no significant difference between the values obtained in both experiments after 18 hours. Substitution of the

air by oxygen has thus no effect on the respiration rate under the prevailing experimental conditions.

Similar measurements were carried out with bulbs, the dry scales of which had been removed. In this experiment too, the rise of the respiration rate after transfer from 25.5° to 20° C in oxygen was as great as that in air. So, it may be concluded that the respiration rate of iris bulbs is not limited by the relatively low oxygen content of the surrounding air.

The rise in respiration rate after removing the dry scales will have to be ascribed to the influence of an as yet not identified stimulus, possibly the loss of water from the first scale.

In the next experiment (Exp. 5) the dry scales and three of the fleshy scales were removed successively. Each time, the respiration rate, both of the fleshy scales and of the remainder of the bulb were determined.

Exp. 5. Respiration rates of the 1st, 2nd and 3rd scale of Wedgwood iris bulbs and of the remaining parts. September 1955.—Respiration rate determined 18 hours after removal of the dry scales.—Storage temperature 25.5° C; temperature after removal of the dry scales 20° C.—Averages of 5 items.—Other data as in Fig. 1.

	fresh weight g	ml CO_2/hr		ml O_2/hr	
		found	calculated	found	calculated
bulb, 2 fleshy scales removed	3.3	0.28		0.33	
2nd scale	4.3	0.08		0.07	
bulb, 1 fleshy scale removed	7.6	0.35	0.36	——	0.40
1st scale	15.0	0.87	0.29	1.05	——
bulb, dry scales removed	22.6	0.64	1.23	0.64	1.45

At the beginning of the measurements, the respiration rate had increased for two reasons, by the change in temperature from 25.5° to 20° C, 18 hours earlier, and by the removal of the dry scales.

Nevertheless, the sum of the respiration rates of all parts is still twice as high as the respiration rate of the whole bulb. This difference is entirely due to the first fleshy scale.

The respiration rate of the isolated scales was again measured 24 hours later, as we had observed earlier (Exp. 3) that it then is at its maximum (Exp. 6).

Exp. 6. The change in respiration rate of the 1st, 2nd and 3rd scale after cutting. Same data as in Exp. 5.

	Immediately after isolation		24 hours later	
	ml CO_2/hr	ml O_2/hr	ml CO_2/hr	ml O_2/hr
1st scale	0.87	1.05	1.66	1.50
2nd scale	0.08	0.07	0.40	0.31
3rd scale	0.03	0.04	0.08	0.08

It may be seen that the respiration intensity of the isolated scales again increased by a factor of 2 to 3. It seems that an activation process takes place in the scales, owing to which the respiration rate reaches a very high level. If this activation is not caused by wounding – and the wounds were very small indeed – it might be due to the same stimulus that plays a rôle in the rise in respiration rate occurring after the removal of the dry scales as shown in Exp. 3. This, together with the effect of the temperature shift, may well account for the entire rise in respiration intensity as observed here.

Another set of experiments was carried out to determine the respiration rate of the isolated bud at different times after removal of the scales (Exp. 7, Fig. 4).

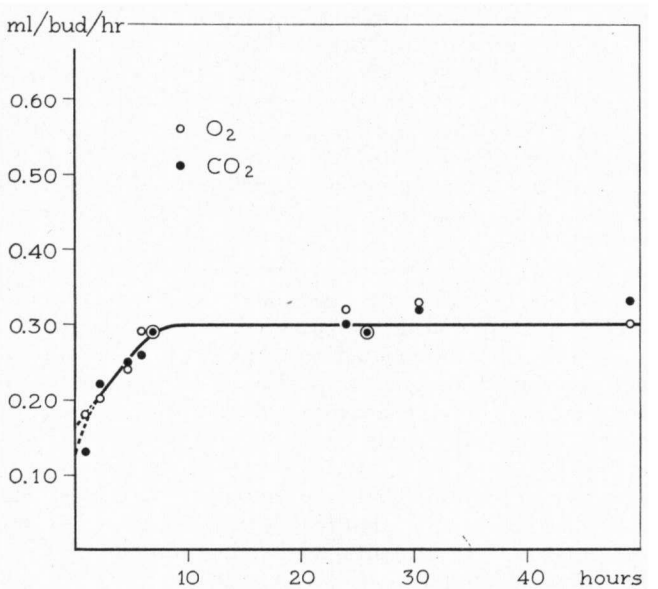

Fig. 4. Respiration rate of an isolated bud (Exp. 7). March 1956.—Isolation of the buds and transfer from 25.5° to 20° C at $t = 0$.—Temperature during experiment 20° C.—Average of 5 buds.—CO_2 production of the whole bulb 0.17 ml/hr; O_2 uptake of the whole bulb 0.18 ml/hr.—Other data as in Fig. 1.

The uptake of oxygen increased within 10 hours to about 2½ times the value it had one hour after the removal of the scales. It was found that the determination of the respiration rate of the bud by extrapolation to $t = 0$ did not yield perfectly reliable results. We always tried to determine the respiration rate as soon as possible after isolation of the buds in order to obtain the best approximation of the desired value. In view of some inevitable variation in the time required for excision, the time of measurement was taken one hour after the start of the isolation procedure in all cases when determination of a time curve was not attempted.

Further experiments showed that loss of water had to be avoided. In an atmosphere with high humidity the oxygen uptake of buds 24 hours after isolation was 50 % higher than in an atmosphere with low humidity.

CHAPTER IV

FLOWER FORMATION AND CARBOHYDRATE METABOLISM

1. Introduction

Many attempts have been made to establish whether floral initiation is correlated with changes in carbohydrate and nitrogen metabolism or in the ratio of organic to inorganic material. One of the first to study this aspect of plant physiology was Klebs who, as early as 1918, put forward the hypothesis that flowering is determined by the balance of organic to inorganic material. In this connection the term "C/N ratio" was introduced by Kraus & Krabill (1918). In later years, however, indications were obtained that changes in C/N ratio are a consequence rather than a cause of floral initiation.

A great number of metabolic processes was studied both in the vegetative and in the reproductive stage of development of the plant in order to establish the crucial process inducing the transition of the growing point from one stage into the next. Numerous valuable results have been obtained, but the problem still remained unsolved. In this part of our work we summarize the results of our investigations on the changes in the carbohydrate content during flower formation in the iris bulb.

In general, investigations as mentioned here are designed in such a way that one of two identical groups of plants is submitted to a flower inducing treatment, while the control group is kept under conditions which prevent flower induction. At pre-set times samples of both groups are analyzed for the contents of certain compounds. The experiments to be recorded were designed according to this method. Samples of scales and buds were separately analyzed.

2. Experimental procedure

The experiments were performed in the autumn of 1955. The summer had been warm, dry and sunny, conditions extremely favourable for cultivating bulbous irises. The bulbs had been obtained from a grower and kept at a temperature above 25° C in wooden boxes, each containing fifty of them. Three series, each of 4 boxes, were used.

The experiments started for the series a and c on September 1st, for series b on the next day. At that time, the rapid fall in respiration rate, always observed after lifting, had not yet stopped completely.

It appeared to be of interest to study the action of three different

treatments on carbohydrate metabolism in connection with flower formation.

Group A was kept at 31° C for two weeks and afterwards for nine weeks at 13° C; we will denote this as 2W 31°–9W 13°.

It is known that this treatment yields the best results with regard to early flowering in practice.

Group B was stored at 2° C for two weeks and afterwards for nine weeks at 13° C (2W 2°–9W 13°). According to BLAAUW, LUYTEN and HARTSEMA (1936) a pretreatment at low temperature has an unfavourable influence on flowering.

Group C was kept at 31° C for two weeks and afterwards for nine weeks at 25.5° C (2W 31°–9W 25.5°). This treatment was included, because flower initiation is inhibited at high temperatures.

Samples consisting of 20 bulbs were analyzed at different times in the course of the temperature treatments.

In order to determine the variability of the samples, CO_2–production and O_2–uptake of 10 samples of 5 bulbs, or of 10 samples of 5 buds, drawn from a lot of bulbs stored at 25.5° C, were determined at 20° C.

The averages and the corresponding standard deviations were calculated. They were found to amount to 0.14 ± 0.01 ml CO_2/hr and 0.16 ± 0.02 ml O_2/hr per bulb and to 0.14 ± 0.02 ml CO_2/hr and 0.18 ± 0.02 ml O_2/hr per bud.

3. EXPERIMENTAL PART

3.1. *The development of the flower primordium in iris.*

BLAAUW (1935) distinguished six stages in the development of the flower primordium of *Iris* cv. "Imperator" (Table I). Often apices are encountered representing an intermediate phase of development.

TABLE I

Stages of development of flower primordia in Iris cv. "Imperator" according to BLAAUW (1935).

Stage	Description	
I . . .	apex egg-shaped; leaves still formed	vegetative stage
II . . .	apex dome-shaped	reproductive stage
III . . .	stamina	" "
IV . . .	outer tepals } each distinguishable as a separate primordium	" "
V . . .	inner tepals }	" "
VI . . .	carpels	" "

Wedgwood iris shows almost the same course of morphological development. So, for the shape of the growing point in the various stages of development of this variety, reference may be made to the drawings in BLAAUW's paper (1935). Photomicrographs of longi-sections of the shoot apex of Wedgwood iris at different stages of flower formation are presented in Plate I.

Bulbs incubated at 13° C form flower primordia after a relatively short period (HARTSEMA & LUYTEN, 1940). In the vegetative shoot apex the following zones can be clearly distinguished (Plate I; A): a two-layered tunica (1) , a central zone of larger cells (2), proximal in the middle the parallel rows of the rib meristem (3), on either side the provascular meristem (4) and, at a greater distance from the axis but still underneath the tunica, young leaf primordia (5). This zonation pattern agrees well with those of *Succisa pratensis* and *Chrysanthemum morifolium* as described by PHILIPSON (1947) and POPHAM & CHAN (1952) respectively.

Twenty-five days after the beginning of the cold treatment the shoot apex was still vegetative, and the transition into the reproductive stage proceeded in about one week. During this period the cells of the central zone were gradually transformed into a mass of small meristematic cells, while the apex increased about 50 % in width (Stage I-II, Plate I; B). Five weeks after the beginning of the treatment the shoot apex was approximately in stage III; the staminal primordia could be clearly distinguished and the primordia of the outer tepals were beginning to differentiate (Stage III, Plate II; A). Two weeks later the inner tepals could be completely distinguished (Stage V, Plate II; B).

3.2. *The morphological development of the shoot*

At the beginning of the experiments, four leaf primordia had already been formed on the growing point. All bulbs of the series 2W 31°–9W 25.5° remained vegetative.

Within 8 weeks, no new leaf primordia were formed; at the end of the experiments, 3 weeks later, two new leaf primordia were developed.

Since developmental processes in iris bulbs are activated by low temperatures, the development of the bulbs of series 2W 2°–9W 13° will start earlier than that of the group 2W 31°–9W 13°. In fact, during the treatment 2W 2°, one more leaf primordium was formed, whereas in the series 2W 31° the growing point had not changed. After six weeks, the apices of the bulbs of both groups were still vegetative; two to three new leaf primordia had been formed. Twelve days later, on the 40th day of the 13° C-treatment, the apices were in the reproductive stage III, and at the end of the experiment the flower primordia were complete.

From the 54th day until the end of the experiment, 7 samples of 5 bulbs were drawn from each group, one every 3-4 days. In the series 2W 2°–9W 13°, 31 out of 33 bulbs were reproductive; in the series 2W 31°–9W 13° all apices appeared to be reproductive.

These figures do not substantiate the opinion that pretreatment at very low temperature exerts a negative influence on flower initiation.

3.3. *The influence of storage temperature on respiration, carbohydrate metabolism and water content*

3.3.1. The first treatment

At the beginning of the experiments, the scales contained, on a fresh weight basis, 0.2 % reducing sugars, 4 % sucrose, 4 % polyfructosides and 16 % starch. The bud contained approximately 0.1 % reducing sugars, 4 % sucrose, 5 % starch and no polyfructosides.

In general, no significant changes occurred in the amounts of the various carbohydrates during storage for two weeks at 31° or 2° C (Figs. 5-7). The respiration rate at 2° C (Fig. 8) decreased during the first week of storage; without doubt this must in part be considered as the continuation of the normal decline in respiration intensity after lifting. At 31° C this fall was intensified; a steady decrease during the whole period was observed.

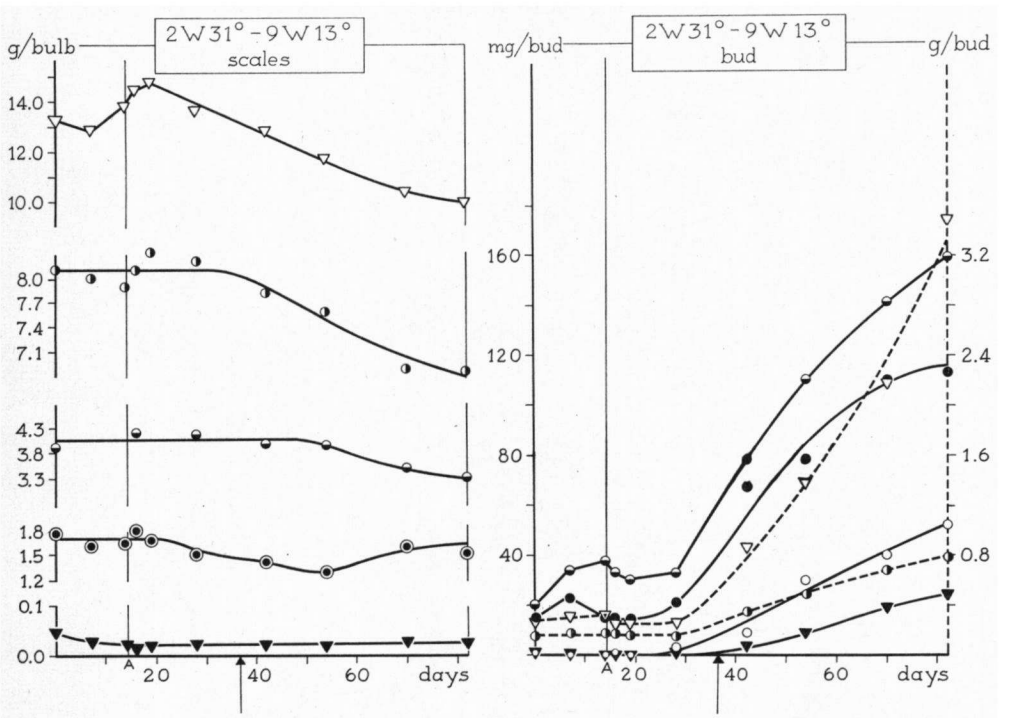

Fig. 5. The content in carbohydrates, dry material and water of scales and buds of the bulbs of Wedgwood iris (Exp. 8). Temperature treatment 2W 31° — 9W 13°; at A the bulbs were transferred from the first to the second temperature. ▽ water, ◑ dry material, ◒ starch, sucrose, ○ polyfructosides, ● sucrose and polyfructosides, ▼ reducing sugars. Carbohydrates values have been recorded as hexoses— ↑ six leaves stage.—Duplicate determinations.

3.3.2. The second treatment

Respiration rate

The respiration rate of the bulbs kept at 25.5° C remained fairly constant during the whole period. In the buds a slight increase of the oxygen uptake was observed between the 40th and the 56th day of the second treatment. In this period two leaf primordia were formed.

When the bulbs, having been stored at 2° C during two weeks, were subjected to the temperature treatment at 13° C, the oxygen uptake increased rapidly. It reached a maximum on the 28th day when 6 leaf primordia were present. Afterwards it decreased.

When the bulbs after storage at 31° C were transported to the thermostat at 13° C, the respiration rate continued to decrease during five days; afterwards it increased during three weeks, the maximum being lower than after a pretreatment at 2° C. At that time 7 leaf primordia were present.

In the buds of the group 2W 2°–9W 13°, the respiration rate was at its maximum on the 40th day of the second treatment (stage

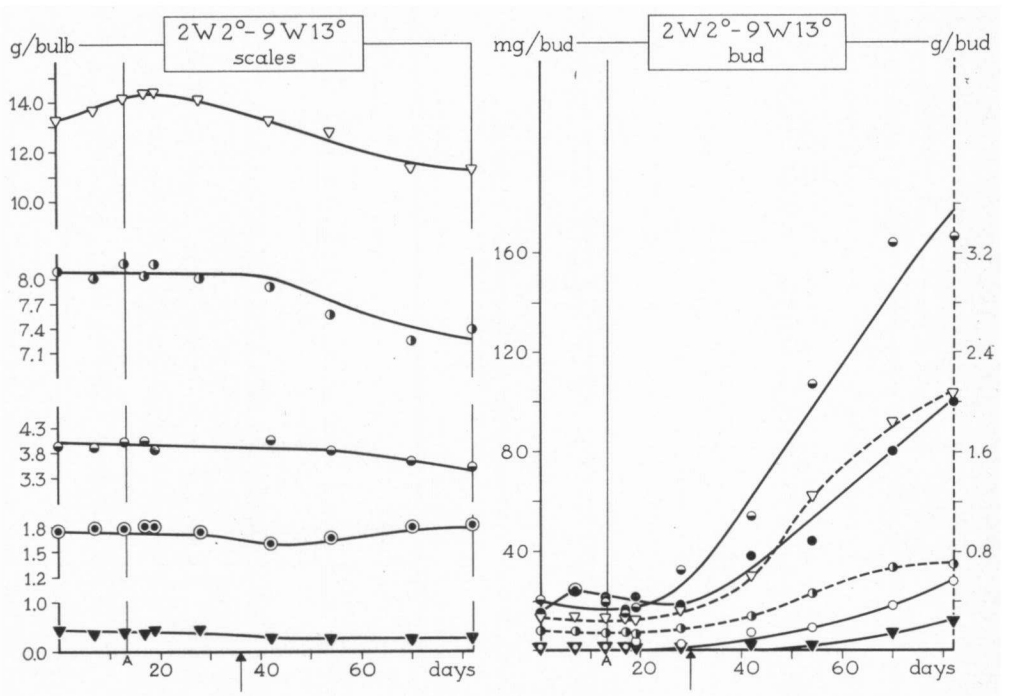

Fig. 6. The content of carbohydrates, dry material and water in scales and buds of the bulbs of Wedgwood iris (Exp. 9). Temperature treatment 2W 2° — 9W 13°. —Other data as in Fig. 5.

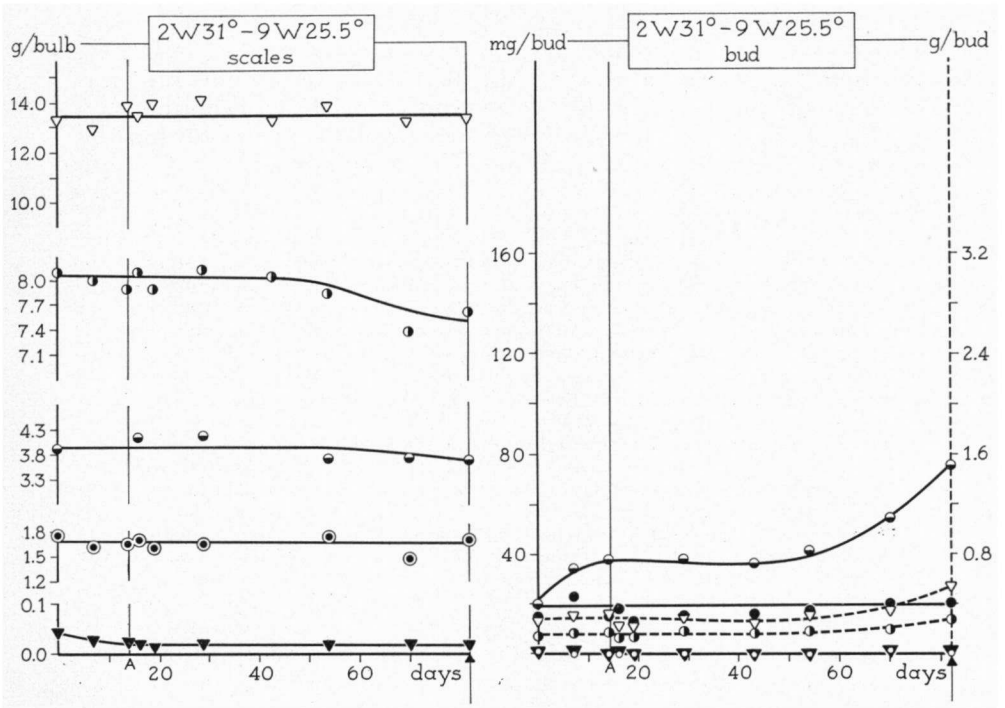

Fig. 7. The content of carbohydrates, dry material and water in scales and buds of Wedgwood iris (Exp. 10). Temperature treatment 2W 31° — 9W 25.5°.—Other data as in Fig. 5.

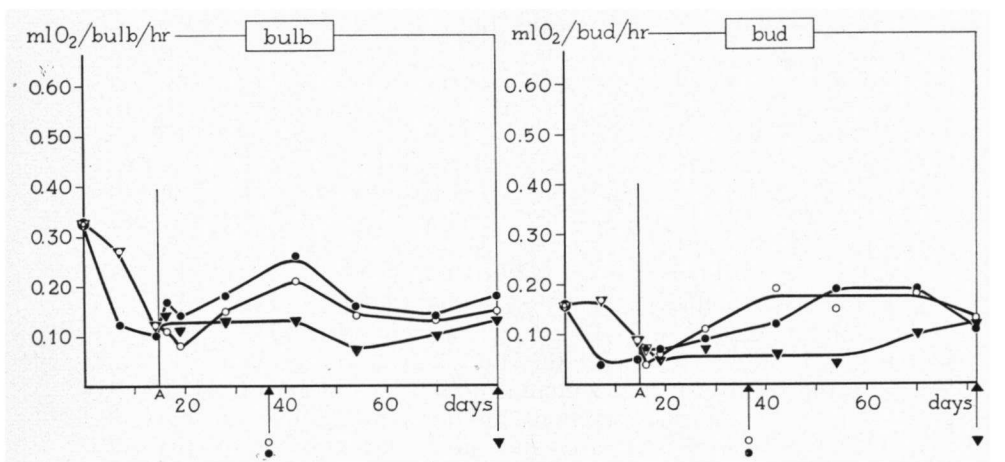

Fig. 8. The effect of different temperature treatments on the oxygen uptake of bulbs and isolated buds of Wedgwood iris (Exp. 11). ○ 2W 31° — 9W 13°; ● 2W 2° — 9W 13°; ▼ 2W 31° — 9W 25.5°.—At A the bulbs were transferred from the first to the second temperature.— ↑ six leaves stage.—Duplicate determinations.

III), in those of the group 2W 31° – 9W 13° on the 28th day. In both cases oxygen uptake decreased slightly after the 54th day. So, both at 13° and at 25.5° C an increase in respiration rate was observed in the period of the formation of the leaf primordia.

Part of the experiments was repeated in 1958 by measuring the respiration rate of the buds during flower initiation at 13° C using the conventional Warburg technique (Exp. 12).

The bulbs were disinfected for 5 minutes in a 1 ‰ mercuric chloride solution to which a few drops of a wetting agent (T-Pol, Shell) had been added, and rinsed in tapwater. The scales were removed, and the stem-discs rinsed once more in tapwater overnight. The next morning all the leaves except the two or three youngest ones were removed and by means of a cork-borer, the central part of the stem-disc carrying the undamaged growing point was cut out. The length of the cylinder was 8 mm, the diameter 5 mm, a little more than the largest diameter of the growing point; fresh weight was about 125 mg. The oxygen uptake of five tips in sterilized Knop solution (diluted 1 : 1) was measured by ordinary Warburg technique. All determinations were continued for 3–4 hours and carried out in duplicate. No indications were obtained that microbial gas exchange interfered with the measurements, the oxygen uptake in the presence and absence of penicillin (up to 400 I.U.) being equal.

When a disc of 2 mm thickness with the shoot apex was cut off from the cylinders the oxygen uptake per 100 mg tissue did not change. The respiration rate of the growing point did not differ much from that of the underlying tissue.

The bulbs of this series were transferred to 25.5° C immediately at their arrival on August 25. From September 23 till November 25 samples of 10 bulbs were transferred with intervals of 3-4 days to a thermostat at 13° C. On November 25 the respiration rate of all samples was determined. In this way, the oxygen uptake was measured of bulbs being stored for periods from ½ till 9 weeks.

After the measurements, the stage of development of the shoot apex was established by means of a binocular microscope. The results are summarized in Fig. 9.

These measurements confirm the earlier results. The respiration rate of the stem-discs increases until the shoot apex becomes reproductive, afterwards it decreases.

There seems to be, however, a second and smaller maximum after 8 weeks at 13°, coinciding with the differentiation of the gynoecium.

Carbohydrates dry weight and water

In the scales of the groups 2W 31° – 9W 13° and 2W 31° – 9W 25.5° the content of reducing sugars, after having decreased from 0.2 % to 0.1 % fresh weight during the storage at 31° C, remained constant during the rest of the experimental period. In the series 2W 2° – 9W 13°, the reducing sugar content did not change during storage at 2° C; about two weeks after the start of the second treatment it decreased to the same level as in the other series.

In the series 2W 31° – 9W 25.5°, the total amount of sucrose,

[1]) In later experiments it could be shown that the peak in the oxygen uptake at this time of the flower formation is real.

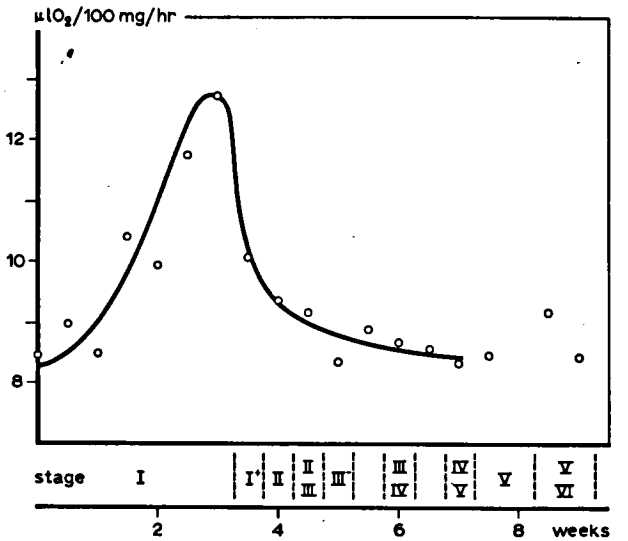

Fig. 9. Respiration rate of stem-discs of iris bulbs during flower initiation (Exp. 12).

polyfructosides and starch remained constant throughout the experimental period. When the bulbs were transferred to 13° C a slight decrease in the total amount of sucrose and polyfructosides could be demonstrated shortly afterwards. A minimum was obtained about the 40th day. Until this day the amount of starch remained constant. Afterwards, the total content of sucrose and polyfructosides increased, whereas the amount of starch decreased.

When the bulbs were stored at 13° and 2° C respectively, the water content of the scales increased during a short time. This may be due to the fact that the relative humidity in both thermostats was higher than in the thermostat of 31° C.

After some days the increase in water content suddenly stopped and changed into a continuous decrease owing to the growth of the bud.

In the group 2W 31°–9W 25.5° practically no change in water content of the scales was observed.

In the series 2W 31°–9W 13° the dry weight of the scales decreased after the 14th day of the second treatment. In the group 2W 2°–9W 13° this decline started later and was less marked; in the group 2W 31°–9W 25.5° the dry weight decreased slightly towards the end of the experiment.

As to the buds, we will compare the results obtained with those which became reproductive with the results obtained with those remaining vegetative in Table II.

From this Table it can be seen that the physiological changes in the buds, connected with the transition from the vegetative into the reproductive state, occur long before the morphological transformation becomes visible by microscopical observation.

TABLE II

Carbohydrate content of buds in the vegetative and reproductive stage as resulting from different temperature treatments.
shoot apex vegetative: —, shoot apex reproductive: +

	a	b	c	d	e	f	g	h	i
1st treatment 2nd treatment	2W 2°	2W 31°	2W 2° 4W 13°	2W 2° 6W 13°	2W 31° 4W 13°	2W 31° 6W 13°	2W 31° 4W 25.5°	2W 31° 6W 25.5°	2W 31° 9W 25.5°
number of leaf primordia	5	4	6	6	7	6	4	5	6
shoot apex	—	—	—	+	—	+	—	—	—
mg/bud									
reducing sugars	1	0	2	2	4	9	0	0	0
sucrose	21	15	38	44	67	78	16	17	20
polyfructosides	1	0	7	9	9	30	0	0	0
starch	19	38	54	107	78	110	36	41	75
water	259	323	596	1243	856	1373	243	311	530
dry material	138	172	269	455	346	482	165	179	268

The bulbs had reached nearly the same stage of development after treatments 2W 2°–4W 13°, 2W 31°–9W 25.5° and 2W 31°–4W 13°. The shoot apex was still vegetative and in the two former series 6 leaf primordia were formed, whereas in the latter seven were present. Comparing the results given in columns c, e and i and taking into account that the development had proceeded after 2W 31°–4W 13° slightly beyond the six leaves stage, it is noticed that a similar developmental stages the chemical composition is practically equal, although the developmental activity is much greater at 13° C than at 25.5° C. Merely the content of soluble carbohydrates was smaller after 9W 25.5° than after 3½ to 4W 13°, but this may be due to the fact that in the former case the bulbs had been respiring for a longer period. It is striking that polyfructosides, constituting about 4 % of the fresh weight of the scales, are absent to the bud at 25.5° and 31° C.

In the second place, we want to discuss the influence of the temperature applied during the first treatment on the development during the second one. In this connection the stem-discs in the group 2W 31°–9W 13° have to be compared to those in the group 2W 2°–9W 13°. The bulbs of the two series became reproductive at approximately the same time and the primordia were complete at the end of the experiment. In general, the accumulation of material in the buds proceeded at a lower rate after treatment 2W 2° than after 2W 31°, and the final values obtained for the amounts of soluble carbohydrates, dry material, and water were lower (cf. Figs. 5 and 6). The increase in starch content was the same in both cases. As for sucrose, the amount per bud was smaller at the end of treatment 2W 2°–9W 13° than after 2W 31°–9W 13° (100 mg as compared to 113 mg); on a fresh weight base, however, it was always larger (4.32 % as compared to 3.51 %, determined on the 82nd day). This may be due to the amount of sucrose increasing during the

cold treatment without a corresponding decrease after the beginning of the second treatment, while the growth rate of the bud is smaller after 2W 2° than after 2W 31°.

So, although the differences observed are only of minor importance, the developmental processes in iris bulbs seem to be more favoured by high than by low temperatures during the period preceding the 13° C-treatment.

The experiments just described deal with the effects of low or high temperature conditions of long duration. The effect of these conditions lasting only a few days were examined too. To this end, bulbs having been stored up to 68 days in thermostats were transferred to quite a different temperature and the changes in the carbohydrate metabolism determined. A few special cases excepted, the results were complicated and could not easily be interpreted. I shall restrict myself to the effect on respiration and metabolism of the polyfructosides in the buds (Exp. 13).

The respiration rate rapidly increased when the temperature was raised and decreased in the reverse condition, Q_{10} being 2-2.5.

Exp. 13. Effect of a change in temperature of relatively short duration on the polyfructoside-content of the bud. All the values are presented as mg hexose per bud.

first treatment	2W 31°			2W 2°			2W 31°		
second treatment	13°			13°			25.5°		
third treatment	31°			2°			31°		
	2nd treatment days	3rd treatment days	polyfructosides	2nd treatment days	3rd treatment days	polyfructosides	2nd treatment days	3rd treatment days	polyfructosides
	0	—	0	0	—	1	0	—	0
	2	—	0	2	—	1	2	—	0
	5	—	0	5	—	2	5	—	0
		1	0		1	3		1	0
		4	0		4	2		4	0
	14	—	3	14	—	2	14	—	0
	40	—	30	40	—	9	40	—	0
		1	23		1	21		1	0
		4	23		4	20		4	0
	56	—	40	56	—	18	56	—	0
	68	—	52	68	—	28	68	—	0
		1	42		1	39		1	0
		4	53		4	37		4	0

It can be deduced from Exp.13 that in experiments of short duration a change in temperature only influences the rate of the polyfructoside metabolism if it is already existing. A rise in temperature decreased the amount of polyfructosides in the buds, a fall in temperature increased their content. The variations in polyfructoside content exceeded those in carbon dioxide production 5-20 times, calculated on equal quantities of C.

4. Discussion.

The respiration rate of bulbs and buds increased early in the period of storage at 13° C; during this period the leaf primordia were formed. At 25.5° C however, the respiration rate of the bud increased too at the end of the experimental period, while new leaf primordia were formed. Therefore, the increased rate of respiration need not be correlated with the transition of the shoot apex into the reproductive state.

At 13° C, the increase in oxygen uptake led to a maximum about the moment the large cells of the central zone of the apex became meristematic, indicating the transformation from the vegetative into reproductive state. It was not possible to decide whether this maximum lies shortly before, at, or shortly after the moment of transition. The fact that the rise in oxygen uptake per bud practically equals that per bulb indicates that the respiration rate of the scales did not increase.

Both in Experiment 11 and Experiment 12, the oxygen uptake per unit fresh weight after 3 to 4 weeks at 13° C is about 50 % higher than it is at the beginning of this treatment. Since in Exp.12 the young leaves were removed before the start of the measurements, it appears that the rise in respiration intensity occurs not only in the developing leaf primordia but also in the basal part of the stem-disc.

As for the content of carbohydrates, under the influence of the 13° C treatment hydrolytic processes are started in the scales, while in the bud soluble as well as insoluble carbohydrates are accumulated. Starting from corresponding stages of development (2W 31°-9W 25.5°, 2W 31°–4W 13°[1]), and 2W 2°–4W 13°), considerable changes in the chemical composition of the stem-disc could be demonstrated after two weeks if the growing points had become reproductive (2W 2°–6W 13° and 2W 31°–6W 13°), but only small changes were observed when the bulbs remained vegetative (2W 31°–11W 25.5°). The latter were determined by extrapolation. (Table III)

Comparing these differences with those tabulated in Table II, we may conclude that the transition from the vegetative to the reproductive state involves a considerable increase in carbohydrates in the bud. That the increase in insoluble carbohydrates is smaller after 2W 31° may be due to the fact that after 2W 31°–4W 13° the development was a little more advanced. As to the greater increase in soluble carbohydrates, especially polyfructosides, it was already noted that certain processes seemed to be more favoured by high than low temperatures during the period preceding the 13° C–treatment. Also at 25.5° C, carbohydrates appear to be transported from the scales to the stem-disc, be it in very small quantities.

Our experiments did not indicate that the metabolism of the bud abruptly changes when the latter becomes reproductive, the increase

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[1]) It was already pointed out that in the second group the development had proceeded a little further than in the two others.

n the various constituents being as large during two weeks previous to the transition as it is afterwards (Fig. 10).

It appears from Fig.10 that the amount of soluble sugars in the buds is greater at 2° C than it is at 31° C. This also holds for the scales. ALGERA (1947), in his extensive work on the influence of

TABLE III

Changes in the carbohydrate content of buds during transition from the vegetative into the reproductive state

first treatment second treatment	2W 2° 4W 13° → 6W 13°		2W 31° 4W 13° → 6W 13°		2W 31° 9W 25.5°→ 11W 25.5°	
shoot apex	vege-ta-tive	repro-duc-tive	vege-ta-tive	repro-duc-tive	vege-ta-tive	vege-ta-tive
	increase mg/bud		increase mg/bud		increase (by extrapolation) mg/bud	
red. sugars	0		5		0	
sucrose	6		11		0	
polyfructosides	2		21		0	
starch	53		32		25	
water	647		517		250	
dry material	186		136		120	

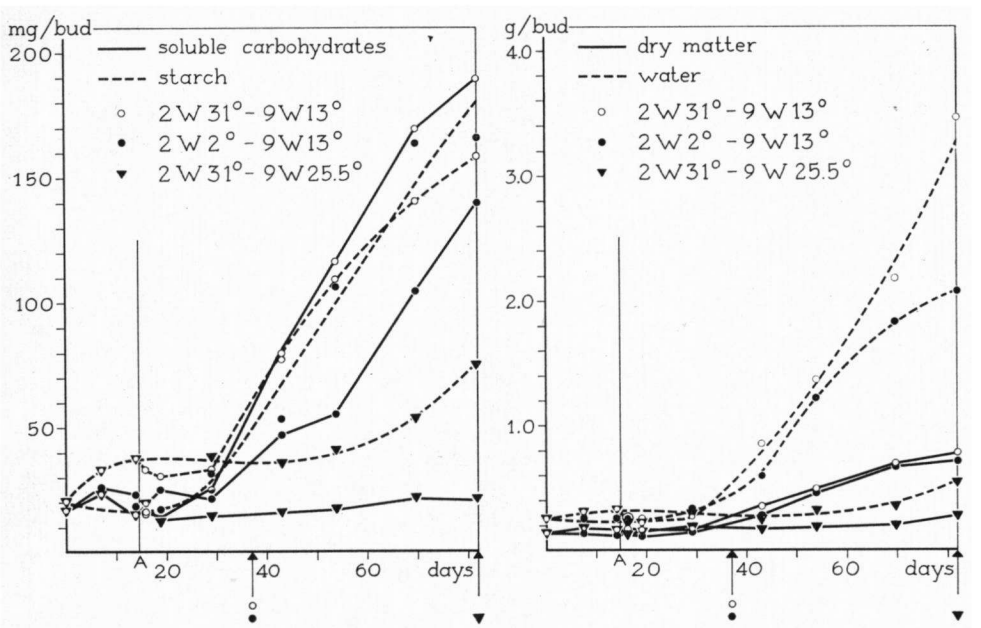

Fig. 10. The influence of various temperature treatments of the bulb on flower initiation and on the content of soluble carbohydrates, starch, dry material and water, of the bud. At A the bulbs were trasferred from the first to the second temperature.— ↑ six leaves stage.

temperature on the carbohydrate metabolism of tulips and hyacinths, stated that the concentrations of sucrose and reducing sugars quickly increased upon cooling, at the expense of the starch present. On the other hand, they were found to decrease quickly when the bulbs or plants were shifted back to the initial temperature. In our experiments, such a ready response was never encountered, except in the case of the polyfructoside content of the bud.

So, on account of the difference in the course of the respiration rate in bud and scales and of the fact that at high temperatures polyfructosides are absent from the bud, the conclusion seems to be justified that scales and bud react relatively independent on temperature treatments.

CHAPTER V

FLOWER INITIATION IN ISOLATED BUDS

1. Introduction

From the foregoing chapter it appears that the metabolic pattern changes considerably in the buds of iris bulbs that have been stored under low temperature conditions causing the transition from the vegetative into the reproductive state at a later stage of development.

One might assume that the origin of these metabolic changes lies in the bud, the function of the scales being merely that of providing material necessary for the growth of the bud, or that the scales act more specifically, e.g. by producing flower forming substances.

We hoped to be able to gain information on this subject by carrying out the following experiments.

2. Methods

The experiments were carried out by culturing excised buds on an agar medium under sterile conditions. Disinfecting the material from which the explants had to be taken proved to be the main difficulty. Satisfactory results were obtained with the following procedure, about 90 % of the explants remaining free from infections. The bulbs were immersed for about 10 minutes in a 1 ‰ solution of mercuric chloride to which a few drops of a wetting agent (T-Pol, Shell) had been added. Afterwards they were washed for a few minutes under running tapwater, and the dry and fleshy scales were removed, except sometimes the median part of the outer fleshy scale. The remaining part of the bulb was disinfected with a freshly prepared, filtered 7 %-solution of calcium hypochlorite. After 30 minutes the material was washed twice during 15 minutes in the sterile tapwater. The small leaves except the two or three youngest primordia were removed with sterile scalpels and forcepses. In some cased only the outer (sheath-) leaves were removed.

The following four types of explants were used in our experiments:

(1) the stem-disc with the growing point and the two or three youngest leaf primordia. This will be indicated as base or (b);

(2) as (1), but all primordial leaves except the sheath leaves still present. This will be indicated as base with leaves or (b + 1);

(3) the excised stem-disc as (1), but the median part of the first fleshy scale still present; indicated as base with scale or (b + s);

(4) the excised stem-disc as (2) with the median part of the first fleshy scale still present; indicated as base with leaves and scale or (b + 1 + s).

The fresh weight of the scale material left in (b + s) and (b + 1 + s) was about ¼ of that of the scales originally present.

The explants were cultured on a medium containing per 1000 ml of pyrex distilled water: sucrose 40 g, $Ca(NO_3)_2.2H_2O$ 500 mg, $MgSO_4.7H_2O$ 125 mg, KH_2PO_4 125 mg, KCl 60 mg, one drop of a 10 %-solution of ferric tartrate, malt extract 2.5 ml, agar 9 g, and 5 ml of a micronutrient solution according to ARNON (1938). The latter contained per 1000 ml of pyrex distilled water: H_3BO_3 572 mg, $MnCl_2.4H_2O$ 362 mg, $ZnSO_4.7H_2O$ 44 mg, $(NH_4)_2MoO_4$ 14 mg, $CuSO_4.7H_2O$ 16 mg, $Co(NO_3)_2.6H_2O$ 16 mg.

The malt extract was prepared according to JANKE (1946); it was sterilized by filtration through a Schott G-5 glass filter and separately added to the medium sterilized by autoclaving.

The pH of the medium was 4.5. Preliminary experiments demonstrated that between the values 4.0 and 6.5, the influence of the pH of the medium on the process of flower formation is very small, its rate being slightly higher at pH 4 to 4.5.

Addition of malt extract was found to exert almost no influence on flower formation.

The stages of development of the shoot apex were denoted with the symbols I through VI (Chapter IV, 3.1). BLAAUW (1935) already pointed out that the periods needed for the development of the successive stages are not equally long. Figure 11 is a time-scale on which these successive developmental stages are recorded.

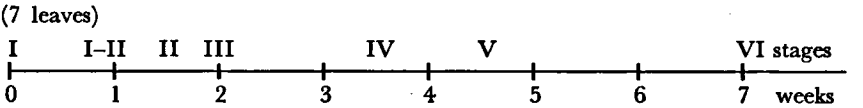

Fig. 11. The duration of the successive stages of development of the flower primordium in bulbs stored at 13° C. Dec. 1957–Febr. 1958. — Two samples a week, each consisting of 5 bulbs. — After 3 weeks at 13° C 7 leaf primordia were developed; the shoot apex then was still vegetative, but started flower formation at the beginning of the 4th week which, therefore, is chosen as $t = 0$.

The total period of 7 weeks, from the beginning until the end of the process of flower formation, was divided into 14 equal intervals

of half a week; the 14 intervals were numbered in order. The results of the culturing experiments will be denoted by these numbers under the heading "score".

The question was raised whether the transition from the vegetative into the reproductive state can proceed in the normal way in an isolated bud. In order to answer this question, bulbs were stored at 13° C. At the end of the fourth week, the shoot apices being vegetative, the buds of a sample were isolated and cultured on the nutrient agar medium at 13° C. It appeared from the study of longitudinal sections that two weeks later both the bulbs and the excised stem-discs had reached stage III-IV. Morphologically, the apices were practically equal (Plate III).

This observation enabled us to investigate the effect of various environmental conditions on the flower initiation of excised stem-discs.

3. Experimental part

3.1. *Storage temperature of the bulbs and flower formation in isolated buds*

The experiment just described was repeated in a more extensive way. The bulbs had been stored for 8 months at 25.5° C and a relative humidity of about 70 % in order to keep them in the vegetative state. In part of the bulbs flower formation was induced by storage at 20-22° C during 9 weeks. At the end of this period the apices were still vegetative. Immediately afterwards the buds were excised and cultured for 6 weeks on nutrient agar (Exp. 14).

Exp. 14. Influence of storage temperature on flower initiation in isolated buds. n_v: number of explants still vegetative at the moment of control; n_r: number of explants being found reproductive at the moment of control. Score: stage of flower formation in reproducing apices in units ranging from 0–14, according to Fig. 11.

Storage temperature of the bulbs until April 15		25.5° C											
Storage temperature of the bulbs from April 15 until June 22		25.5° C						20°–22° C					
		n_v 15	n_r 0	score —				n_v 7	n_r 0	score —			
		Buds isolated on June 22											
Incubation temperature		25.5° C			13° C			25.5° C			13° C		
Preparation	Explants incubated during	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score
(b)	4 weeks	—	—	—	—	—	—	0	2	9	0	2	7
(b)	6 weeks	4	0	—	4	0	—	—	—	—	—	—	—
(b + 1)	6 weeks	3	0	—	4	0	—	—	—	—	0	3	8

From Exp. 14 it appears that the stem-discs excised from bulbs that have been stored at 25.5° C throughout, have not become reproductive by culturing at 13° C during 6 weeks. If they had not

been removed from the bulbs, they would quite certainly have formed flower primordia. If, however, the flower formation had previously been induced by storage of the bulbs at 20°-22° C, flower primordia would have developed afterwards in the excised buds.

In the following pages we will denote a storage at low temperature conditions leading to the induction of the reproductive state as a "pretreatment". A short treatment of the bulbs at 13° C appeared to suffice. Care was always taken that the pretreatment never led to primordial stages beyond the vegetative state (stage I). Unless otherwise indicated the duration of the experiments included both the time of pretreatment and the period during which the explants were incubated at the temperature stated.

At this point the average duration of the pretreatment required for floral induction in the isolated stem-disc was determined. In addition we investigated whether this duration depends on storage time. In Exp. 15 results are presented obtained with samples incubated after varying periods of storage and pretreatment.

Before discussing the results of this experiment it should be pointed out that in the beginning of the season the transition from the vegetative into the reproductive state takes about 5 weeks (pretreatment period included); at the end of the season it certainly takes less than 4.5 weeks. When the isolated stem-disc had not become reproductive at the end of 6 and 5 weeks respectively it did not proceed to flower formation any more.

Shortly after digging, only part of the growing points will develop flower primordia under favourable conditions; later in the season the percentage of reproductive apices increases independently of the pretreatment applied. On the other hand, the shorter the storage period at 25.5° C, the longer the period of pretreatment at 13° C required. After a two weeks' storage period, the minimum duration of the pretreatment is 4 weeks. Three months later it is about one week, perhaps even less. A pretreatment can never be completely omitted for explants consisting of the basis alone without the young leaves (b). Both factors, the storage and the pretreatment, influence not only the number of apices becoming reproductive (n_r) but also the rate of formation of the flower primordia (score).

Similar experiments have been carried out with isolated stem-discs the young leaves of which had not been removed (b + 1). In general, the same results were obtained as with the bases (b). Flower initiation occurred only if, previous to the isolation of the buds, the bulbs had been stored at a temperature below 25.5° C during one to four weeks.

A remarkable result was obtained with stem-discs with leaves (b + 1) isolated from bulbs having been stored at 25.5° C for many months. Pretreatment appeared no longer needed; in about 6 weeks, cultured on an agar medium at 13° C the buds became reproductive.

Other preliminary experiments revealed that the primordial leaves were able both to promote and retard flower formation depending on the pretreatment applied. More detailed experiments will be discussed in the next section.

Exp. 15. Influence of the period of storage and pretreatment on flower formation in excised buds of Wedgwood iris. Isolated stem-disc (b).—Incubation temperature 13° C.—Other data as in Exp. 14.

Storage period at 25.5°	Total period of pretreatment and incubation (weeks)	No pretreatment			Pretreatment											
					1W 13°			2W 13°			3W 13°			4W 13°		
		n_v	n_r	score	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score
2 weeks	7				2	0	—				2	0	—	0	2	4
1 month	6													0	17	6±0.5
5 weeks	7				15	0	—	5	7	6±1.2	0	14	6±0.5	0	17	10±0.8
2 months	6										0	5	5±0.6			
3 months	7–8													0	10	12±0.5
3 months	9	13	0	—	7	3	6±1									
4.5 months	6				4	6	5±0.8	1	13	12±0.5						
7 months	6	12	0	—												
8 months	5–6				2	10	10±0.9									

TABLE IV

The influence of primordial leaves and scales on the rate of development of the flower primordia in excised buds of Wedgwood iris Bulbs of Exps. 16, 17, 18, 19 and 23 size 10½–11; bulbs of Exps. 20, 21 and 22 size 7–8.—Other data as in Exp. 14. Before the beginning of the experiments the bulbs had been stored at 25.5° C.

Exp. No.	Date	Pre-treatment	Incubation		Explants												Intact bulbs in dry storage		
			temperature	duration (weeks)	(b)			(b + 1)			(b + s)			(b + 1 + s)					
					n_v	n_r	score	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score	n_v	n_r	score
16	Febr. '57	1W 13°	13°	4–5							0	5	8±0.6	0	4	7±0.9	0	8	8±0.4
17	Apr. '57	1W 13°	13°	4	1	6	11±0.6	0	7	4±0.4							0	7	5±0.8
18	Febr. '57	2W 13°	13°	4–5	1	9	12±0.4	0	11	7±0.6							0	8	9±0.5
19	Dec. '56	4W 13°	13°	3	0	5	11±1.0	0	6	8±0.2	0	5	12±0.6	0	6	11±0.7	0	10	9±0.4
20	Apr. '57	4W 13°	13°	3	0	5	7±1.0	1	3	4±0.7	1	4	10±1.0	0	5	9±0.3	0	5	5±0.5
21	Apr. '57	4W 13°	13°	5	1	3	13±0.4	2	3	5±1.9	1	5	14±0.1	0	6	13±0.1	0	6	10±0.5
22	Febr. '57	5W 13°	13°	4–5	0	6	13±0.2	0	11	9±0.5	0	10	14±0.1	0	7	13±0.7	0	8	10±0.3
23	Apr. '57	3W 13°	25.5°	3	0	6	14±0.1	0	5	9±0.5	0	5	14±0.1	0	5	9±1.2	0	4	4±0.1
Totals (Exp. 23 excepted)					3	34	11±0.4	3	41	7±0.4	2	29	12±0.4	0	28	11±0.5	0	52	8±0.3

3.2. *The influence of primordial leaves and scales*

In the preceding section discussion has been restricted to flower initiation in excised buds after elimination of the scales. It was found that, under certain conditions, the presence of primordial leaves may promote flower initiation. As similar effects can be expected from the presence of the scales, in this section the influence of young leaves and scales on the rate of development of the flower primordia and on floral induction will be investigated.

3.2.1 *The influence of primordial leaves and scales on the rate of development of the flower primordia*

In a discussion of flower initiation we must distinguish between the induction of the reproductive stage and the rate of development of the flower primordia on the reproductive apex. The influence of a factor on the rate of development can only be studied if the pretreatment allows the shoot apex to become reproductive. If the factor concerned is present, it either increases or decreases the rate of development of the flower primordium.

In the experiments, summarized in Table IV, pretreatments were applied ranging from 1 to 5 weeks, dependent on the preceding storage period at 25.5° C, so that in explant (b) an average score of 11, agreeing with BLAAUW's stage V, was obtained.

The rate of development of the flower primordia is practically equal in the explants (b), (b + s) and (b + 1 + s). Even in Exps. 19, 20, 21 and 22, the differences are not significant. From these results it may be deduced that the wound caused by the elimination of the primordial leaves in excising the explants (b) and (b + s) does not decrease the rate of development of the flower primordia. In the explants with leaves (b + 1), however, the score is significantly lower than in (b), so that it is evident that the primordial leaves exert a retarding influence on the development of the flower primordia. This inhibiting effect could not be demonstrated in the presence of the scales.

3.2.2. *The promoting influence of primordial leaves and scales on floral induction*

If one wishes to investigate whether a certain treatment stimulates induction of the reproductive stage in the buds, the bulbs have to be subjected to a pretreatment just not bringing about flower induction ($n_r=0$). A promoting factor then will cause floral induction. If, however, one expected the treatment to inhibit the induction of the flower primordia, the pretreatment should have been carried out in such a way that flower induction would just ensue. An inhibiting factor then will prevent flower formation. Only the former case has been investigated in the following experiments

In Table V examples of the promoting effect of the young leaves are summarized. The experiments were performed in four different years after various storage periods at 25.5° C.

TABLE V

The promoting effect of primordial leaves on flower induction in excised stem-discs. Incubation temperature 13° C.—Other data as in Exp. 14.

Exp. No.	Date	Pre-treatment	Duration of incubation (weeks)	Explant					
				(b)			(b + 1)		
				n_v	n_r	score	n_v	n_r	score
14	June '56	none	6	4	0	—	4	0	—
24	April '57	none	6.5	12	0	—	4	8	6 ± 0.8
25	March '58	none	6	13	0	—	15	14	3 ± 0.3
26	Jan. '59	4D 13°	7	19	0	—	17	7	2 ± 0.2
27	March '59	none	7	33	0	—	10	19	3 + 0.2
Totals				81	0		50	48	

The promoting effect of the young leaves on flower induction is significant at the 0.1% level.

The removal of the primordial leaves inhibited the normal development and no flower primordia were formed. It has to be considered whether this is not due to the apex being so much damaged by the various manipulations that it is no longer able to functionate. From the results summarized in Table IV, however, it was inferred that removal of the young leaves did not decrease the rate of development of the flower primordia after induction. Moreover, the two leaf primordia always left on the shoot apex, use to elongate and may reach an average length of 50 mm (Exp. 26), whereas also new leaf primordia are being formed. So we get the impression that in the experiments presented in Table V some factor is lacking in the explants (b). This resulted in preventing flower induction. On the other hand, about 50 % of the isolated stem-discs that had kept their leaf primordia were able to reach the reproductive stage when incubated at 13° C.

It can be deduced from these experiments that the primordial leaves are concerned in the process of floral induction.

Experiments in which the promoting effect of the scales on flower induction was investigated are presented in Tables VI and VII. We concluded that there is a significant difference between the explants with and without a piece of scale.

In Exp. 32 (Table VII) the buds with young leaves (b + 1) did not become reproductive whereas in Table V it was found that the number of reproductive explants amounted to almost 50 % of the total number of explants. This is due to the fact that the bulbs used in Exp. 32 were so small (size 5 – 6) that their flowering capacity was very much restricted anyhow; in the experiments presented in Table V only well flowering bulbs of size 10½ – 11 were employed.

From the experiments, discussed in this section, it can be concluded that the induction of the reproductive stage is promoted both by the primordial leaves and by the scales.

If the isolated stem-discs, incubated at 13° C, formed flower

primordia, they used to develop in a perfectly normal way. At 25.5° C, however, abnormal primordia sometimes appeared after induction at 13° C.

It appeared from Table IV that the rate of the development of the flower primordia is greatest in the explants (b), (b + s) and (b + 1 + s) cultured on an agar medium, and less in intact bulbs in dry storage. If, after pretreatment at 13° C, during which the reproductive stage is just attained, dry bulbs and explants (b + 1 + s) were incubated at 25.5° C, the difference in rate of development was still greater (Table VIII).

TABLE VI

The promoting effect of the scales on flower induction in excised buds without young leaves. Incubation temperature 13° C.—Other data as in Exp. 14.

Exp. No.	Date	Pre-treatment	Duration of incubation (weeks)	Explant					
				(b)			(b + s)		
				n_v	n_r	score	n_v	n_r	score
28	March '57	none	6	12	0	—	0	10	12 ± 0.6
29	Dec. '57	none	9	13	0	—	0	8	14 ± 0.1
Totals				25	0		0	18	

TABLE VII

The promoting effect of the scales on flower induction in excised buds with young leaves. Bulbs of Exps. 30 and 31 size 10½–11; bulbs of Exp. 32 size 5–6.—Other data as in Table VI.

Exp. No.	Date	Pre-treatment	Duration of incubation (weeks)	Explant					
				(b + 1)			(b + 1 + s)		
				n_v	n_r	score	n_v	n_r	score
30	March '57	none	6	3	3	5	0	6	13 ± 0.8
31	Dec. '57	none	6	8	1	3	0	9	13 ± 0.2
32	May '57	none	10	8	0	—	4	3	12 ± 1.2
Totals				19	4		4	18	

TABLE VIII

The rate of the development of the flower primordia in isolated stem-discs, compared with that in intact bulbs in dry storage. Incubation temperature 25.5° C.—Other data as in Exp. 14.

Exp. No.	Date	Pre-treatment	Duration of experiment (weeks)	Bulb in dry storage			Explant (b + 1 + s)		
				n_v	n_r	score	n_v	n_r	score
33	Dec. '56	5W 13°	7	0	5	6 ± 1.0	0	10	11 ± 0.7
34	Dec. '57	4W 13°	7	8	11	4 ± 0.2	0	13	7 ± 0.8
Totals				8	16	5 ± 0.1	0	23	9 ± 0.2

4. The influence of some substances

In order to investigate further the nature of the factor promoting flower induction, the effect of addition of various substances to the culture medium was tested.

4.1. *The influence of sucrose and other sugars*

As was stated in Chapter IV, changes in the amount of soluble carbohydrates occur in the stem-disc during the process of flower induction. Therefore, sucrose, dextrose, inulin, laevulose, galactose and maltose (4 %) were added to the medium. It appeared that neither the process of flower induction nor the rate of development of the flower primordia was affected. Only lactose had a slightly inhibiting effect.

In order to rule out the possibility that these sugars were insufficiently taken up from the culture medium, the amounts of reducing sugars and sucrose in the stem-disc were determined at the beginning and at the end of a nine weeks incubation period at 13° C. It appeared that the isolated stem-disc had absorbed a fairly large amount of sucrose, half of which was converted into reducing sugars. The total amount of them in the isolated bud, was even greater than in the corresponding part of the intact bulb stored under stimilar conditions.

4.2. *The influence of growth substances*

The first compound among the growth substances of which it seems of interest to investigate the influence on flower induction is gibberellic acid. Initially, the gibberellins were introduced as compounds promoting or inducing stem elongation (Stowe & Yamaki, 1957). In later years, however, instances gradually were found of gibberellins promoting flower induction (Phinney & West, 1960).

We investigated the influence of gibberellic acid (GA) on flower induction and on the rate of development of the flower primordia in excised buds with and without leaves. Fifty milligrams of GA [1]) were solved in 1.8 ml absolute ethanol and the solution diluted with pyrex distilled water to 25 ml. The solution was sterilized by filtration through a Schott G-5 glass filter. Different quantities were added to the complete medium after this had been autoclaved. The results of these experiments are given in Tables IX and X. The promotive effect of gibberellic acid on flower induction in explants (b) is significant at the 0.1 % level. There is no significant effect of gibberellic acid on flower induction in explants (b + 1).

From these experiments it is evident that GA has a promoting influence both on flower induction and on the rate of development of the flower primordium. The latter increases with increasing

[1]) The gibberellic acid was kindly put at our disposal by Plant Protection Limited, Fernhurst Research Station, Nr. Haslemere, Surrey, England.

concentrations between 0 and 25μg/ml. No inhibition occurs at concentrations between 25 and 100 μg/ml.

In several higher plants the presence of gibberellin-like substances has already been demonstrated (WEST & PHINNEY (1959); HARADA & NITSCH (1959)). On account of our findings it seems likely that also in iris flower initiation is regulated by such compounds.

The promotive effect of GA on flower induction could only be observed in explants without primordial leaves. The rate of development of the flower primordia in explants (b + 1) was only little or not at all increased.

Kinetin whether or not combined with different concentrations of indoleacetic acid (IAA) had no influence on flower initiation. IAA itself inhibited flower induction at concentrations down to 10^{-2} μg/ml, and had no effect at lower concentrations.

Casein hydrolysate and several of the B-vitamins exerted no influence on flower formation.

TABLE IX

The influence of gibberellic acid on flower induction in excised buds of Wedgwood iris. Data as in Exp. 14.—Incubation temperature 13° C.

Exp. No.	Date	Size	Pre-treatment	Duration of experiment (weeks)	Explant	GA μg/ml	n_v	n_r	score
35	Febr. '59	10½–11	4D 13°	7	(b)	0	19	0	—
						40	1	21	9 ± 0.4
36	Jan. '60	6–7	4W 13°	12	(b)	0	10	1	4
						25 or more	16	18	9 ± 0.8
37	Febr. '59	10½–11	4D 13°	7	(b + 1)	0	17	7	2 ± 0.2
						40	28	1	3
38	April '59	10½–11	none	7	(b + 1)	0	10	19	3 ± 0.2
						40	0	17	6 ± 0.4
			1W 13°	7	(b + 1)	0	0	28	4 ± 0.3
						40	0	29	4 ± 0.3
39	Jan. '60	6–7	4W 13°	12	(b + 1)	0	14	1	9
						25 or more	33	7	11 ± 0.7

TABLE X

The influence of gibberellic acid on the rate of development of the flower primordia in excised buds of Wedgwood iris. Explant (b).—Duration of Exps. 7 weeks.—Incubation temperature 13° C.—Other data as in Exp. 14.

Exp. No.	Date	Pretreatment	GA μg/ml	n_v	n_r	score
40	Dec. '58	2W 13°	0	5	8	3 ± 0.2
			10	0	10	6 ± 0.8
			25	0	18	8 ± 0.4
			50	0	17	7 ± 0.5
			100	0	20	9 ± 0.4
41	Jan. '59	1W 13°	0	4	16	4 ± 0.4
			40	0	20	7 ± 0.5

5. Further investigations on the influence of the scales on flower induction

In 3.2 it was demonstrated that scale tissue promotes flower induction in isolated stem-discs. In order to investigate whether this effect is due to a material agent, isolated buds were incubated together with an isolated fragment of the scales ((b) + (s)). The size of the fragment was equal to that in explants (b + s), i.e. about ¼ of the total mass of the scale tissue. As controls, excised stem-discs with (b + s) or without (b) a fragment of the scales were employed. The presence of an isolated scale fragment promoted the process of flower induction; the difference being significant at the 0.1 % level (Table XI).

TABLE XI

The influence of detached fragments of scales on floral induction in isolated stem-discs of Wedgwood iris. (b) + (s): a detached fragment of scale with its cut surface inoculated onto the agar medium beside the excised bud.—Incubation temperature 13° C.—Other data as in Exp. 14.

Exp. No.	Date	Pretreatment	Duration of experiment (weeks)	Explants								
				(b)			(b) + (s)			(b + s)		
				n_v	n_r	score	n_v	n_r	score	n_v	n_r	score
42	Apr. '57	none	5	12	0	—	4	6	6±1.0	0	10	12±0.6
43	Jan. '58	none	9	13	0	—	7	2	7	0	8	14±0.1
44	Oct. '59	2W 13°	7	13	2	4	9	14	4±0.5			
Totals				35	2		20	22				

We can interpret these results by assuming that a compound, present in the scales, after having been transported to the shoot apex, caused the transition from the vegetative state into the reproductive one.

Finally, it was investigated whether aqueous extracts of scales and stem-discs of bulbs stored at 25.5° or at 13° C possessed any flower inducing power on excised buds (b). The extracts were added to the culture medium in quantities equivalent to one or a quarter of a bulb; in the nutrient medium of the control series an amount of sucrose was incorporated equivalent to the sucrose content of the extract. No difference was observed between the stem-discs cultured on agar media with and without extract.

GENERAL DISCUSSION AND SUMMARY

I

In the vegetative state the rate of cell division in the apex of Wedgwood iris is very slow. The first sign of the transition from the vegetative into the reproductive state is an increase in the rate of cell division in the rib meristem, about 10 cell layers below the median tunica, as appears from the photomicrographs,

presented in Plates I and II. The cell division activity gradually spreads over the entire apex. At the same time, the zonation pattern, characteristic for the vegetative phase, vanishes. A slow and gradual transition from one phase into the next is observed. The reproductive apex can be described as a parenchymatous core enclosed by a meristematous mantle. This is in accordance with the picture given by GRÉGOIRE (1938) for several reproductive apices. We cannot accept, however, his view that a reproductive apex differs from the vegetative one in many respects.

II

After the bulbs have been incubated at 13° C for about 4 weeks, the growing points become reproductive. During this period the respiration rate of the bulb increases, afterwards it decreases. The maximum lies about 50 % above the initial value (Exps. 11 and 12). The course of the respiration intensity is restricted to the bud, the oxygen uptake remaining constant in the scales. The rise and fall equally occur in the young developing leaves and in the shoot apex with the stem-disc proper. So far, nothing can be said about the nature of the increase that strongly reminds of the climacteric peak in ripening fruit. Perhaps after the transition into the reproductive state, respiration is partly mediated by another glycolytic pathway. Experiments to test this possibility are in progress.

Bulbs stored for a period of 9 weeks at 25.5° C and those stored for 3½-4 weeks at 13° C formed two new leaf primordia. The content of carbohydrates, dry material and water of the buds was almost equal in both samples (Table II). Obviously, the carbohydrate content of the bud at a given vegetative stage is independent of the temperature and of the period of storage of the bulbs. If the bulbs are incubated at 13° C, the shoot apex reaches the reproductive stage III in the course of the next fourteen days. During this period, the increase in carbohydrate content proceeds at the same rate as before (Fig. 10). As for this, the moment of the transition is not marked by a sudden change.

Neither the increase in soluble nor that in insoluble carbohydrates seems to be correlated with the appearance of the reproductive stage. It could be shown by adding sucrose to the culture medium, that the content of soluble carbohydrates in the excised vegetative bud can be raised to the level of that in the bud in the intact bulb at the transition stage, while the shoot apex remains vegetative.

III

Although bulbs stored at 25.5° C never become reproductive, a limited flower inducing action is present at this temperature (Exp. 15). It seems that the conditions for flower induction, which in fact arise also at high temperatures, cannot be realized there owing to inhibiting factors becoming more important under these conditions.

Floral induction is promoted by the presence of primordial leaves (Table V) and by a factor present in the scales (Table VI). The latter agent can diffuse from the cut surface of the scale to the excised bud via an agar bridge (Table XI).

It seems possible that a competition exists between the growth process in the young leaves already initiated and that in the flower primordium. Since flower formation is not influenced by reducing sugars, it is not probable that competition for these compounds is at the root of the retardation of flower formation by the young leaves. It seems more conceivable that it is a matter of competition

for growth substances, e.g. gibberellin-like substances, which are of importance for both leaf growth and the development of the flower primordium, in which competition the leaves always take the better part. This might explain not only why on a medium without GA, the rate of development of the flower primordium in explants (b + 1) is smaller than in explants (b), but also why on a medium with GA flower induction is promoted in explants (b) and not in explants (b + 1).

It is also conceivable that in the course of their development the young foliage leaves produce a substance or substances that have an inhibitory effect on the flower forming processes in the apex. Indoleacetic acid, that, as we saw, very much inhibits flower formation, might be such a substance.

In our opinion the preparation of extracts of leaves and buds and the analysis of these extracts both for flower forming and growth substances, would mark a first step in the direction of a solution of this problem. Such investigations are now in progress.

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