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EFFECT OF LIGHT AND GROWTH SUBSTANCES ON FLOWERING OF IRIS × HOLLANDICA CV. WEDGWOOD

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SUMMARY

In the winter period with prevailing low light intensities and short days, the forcing of *Iris* "Wedgwood" can easily result in flower bud abortion (bud blast). Consequently a high proportion of plants fail to produce flowers.

To elucidate the cause of bud blast, plants were subjected to various treatments —$^{12}CO_2$, $^{14}CO_2$, growth substances — in the period of rapid stem elongation about two weeks before flowering.

Plants grown under weak illumination and normal CO_2 concentration (leading to 57% flowering) were compared with plants grown under strong illumination at low CO_2 concentration (leading to 100% flowering). As the dry weight of whole plants of both these groups was almost the same at flowering, bud blast under low illumination cannot simply be attributed to a lack of recent photosynthetic products. The distribution pattern of photosynthates appears to be changed.

It is suggested that a hormone imbalance plays a role in the distribution pattern and consequently in bud blast, since injections of cytokinins, such as N^6-benzylaminopurine, zeatin, and kinetin, during dark treatment can increase the flowering proportion.

1. INTRODUCTION

It has long been known that bud blast is a common failure during early and late forcing in bulbous irises. It is associated with many factors such as kind of cultivar, bulb size, conditions during storage and after planting (BLAAUW 1934, 1935, 1941; BLAAUW et al. 1936a, 1936b; FORTANIER & VAN ZEVENBERGEN 1973; HARTSEMA 1961; HARTSEMA & LUYTEN 1953, 1955a, 1955b, 1961; KAMERBEEK & BEIJER 1964; WALLA & KRISTOFFERSEN 1969). After planting, the most important environmental factors inducing bud blast are insufficient light, high temperature, and shortage of water.

HARTSEMA & LUYTEN (1961) studied light requirements of the iris "Wedgwood" and concluded that flowering is proportional to the amount of light during growth. They supposed that, with low light intensity, reserve materials of the bulb are exhausted before flowering starts. Consequently, in the later stages of development, more recently formed products of photosynthesis (and therefore more light) would be needed to permit the final elongation of flower

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stems and flowers. They showed that additional light for early forcing in greenhouses is most effective if given 1 or 2 weeks before flowering.

More recently, FORTANIER & VAN ZEVENBERGEN (1973) established that the period of greatest stem elongation coincided exactly with the period in which the incidence of bud blast is most strongly affected by illumination.

These experimental results make it clear that illumination during the stage of largest stem elongation is very important for flowering. However, the relation between carbohydrates and flowering is less clear, since no distinction is made between carbohydrates mobilized from various plant organs and recently formed photosynthates, and between distribution pattern of mobilized or recently formed products.

In this study the relation between light conditions and distribution of photosynthates during rapid growth of stem and flower has been investigated. Effects of growth substances are studied too, since growth hormones affect flowering of many plant species.

2. MATERIALS AND METHODS

2.1. Plant and culture

Bulbs of *Iris* × *hollandica* 'Wedgwood' were obtained from a commercial grower. Before planting the temperature treatment of the bulbs for forcing and retarding was carried out by a bulb grower under the following conditions.

After digging, bulbs were stored at 35 °C for two weeks, then at 40 °C for 3 days and finally at 30 °C until 6 or 9 weeks before planting. During the last period treatment was at 9 °C. Two plants were put in each pot (12 cm in diameter, 11 cm high), filled with commercial potting soil. The plants were grown for one week at 15 °C and then for about 5 weeks at 18 °C in a greenhouse.

After this period the plants were transferred to a growth chamber. Daylength was 12 h and irradiation under standard conditions was 56 W m^{-2} in the spectral region 400–700 nm (10% from incandescent lamps, 90% from high-pressure mercury-vapour lamps with fluorescent emission, Philips HPL). Air temperature and air humidity were kept at 18 °C and at about 80% r.h., respectively, during day and night.

2.2. Light and carbon dioxide experiments

Control plants were subjected to standard conditions as described under 2.1 (56 W m^{-2} and 0.03% CO_2) during the whole growing period in the chamber. Treatments (low light, dark, low CO_2 concentration) were given around the light-sensitive period (see under results 3.3.) of rapid stem elongation in control plants, about 35–38 days after planting and about 14 days before the onset of detectable flowering.

In low light and in low CO_2 experiments, 12 plants were placed in a translucent Plexiglass box (100 cm × 50 cm × 65 cm). The box was placed in the growth chamber. Low-light treatment (2.6 W m^{-2}) was obtained by covering the box with cheesecloth. Day and night temperature inside the box, under standard

conditions, was 21 °C and 19 °C, respectively.

Normal CO_2 concentration (0.03 %) was approached by flushing the box with air from the growth chamber. The low CO_2 concentration (about 0.003 %) was obtained by flushing the box with air that had first passed through a column of soda lime. The standard light intensity in the box was 48 W m^{-2}. The CO_2 concentrations in the box were measured with a Wösthoff Absolute CO_2 Analyser.

In most experiments low CO_2 treatments were not included. Then the box was not used and the low-light treatments were obtained by placing cheesecloth 70 cm over the plants.

2.3. Administration of labelled carbon dioxide to a leaf

Radioactive CO_2 was introduced into a glass tube 19 cm long and 1.5 cm in diameter containing the second leaf (from the base) or sixth leaf, (in total 7 leaves) as described by VAN DIE & TAMMES (1964). The tube was kept around the leaf for 1 h. Sodium [^{14}C] carbonate (20 μCi per plant) was used for liberation of $^{14}CO_2$.

2.4. Administration of labelled phosphate into a 2nd leaf

A small area (1 mm^2) was stripped with a razor blade (to obtain better penetration) and surrounded by lanolin paste. One drop of the isotope solution (0.3 μCi of $Na_3{}^{32}PO_4$) was administered into the hole and kept there during the experiment (24 h).

2.5. Preparation of samples for counting

After harvesting, plants were carefully washed and separated into different parts. After measuring fresh weight, they were dried in a stove at 90 °C for 48 h. The dried material containing ^{14}C was ground in a mortar and ashed, using the dry combustion technique (Berthold Frieseke micro-mat 5010) as described by WEGNER & WINKELMANN (1970). Plant organs containing ^{32}P were extracted with 80 % aqueous ethanol (v/v) and the residue was washed with 80 % ethanol. Filtrate and washings were combined and samples counted in a liquid scintillation spectrometer.

2.6. Estimation of carbohydrate

Total carbohydrate was estimated enzymically, as described by BERGMEYER & BERNT (1970). All enzymes used were of Boehringer quality (Boehringer, Mannheim).

2.7. Experiments with growth substances

One ml of an aqueous solution of the plant growth substance (pH 6.5) was injected into the space between flower and spathe on the 3rd day of the 7 days dark treatment. The following substances were used:

Benzyl-N^6-aminopurine (BAP), zeatin, adenin, kinetin, 6-(γ, γ-dimethylallylamino)purine (2iP), gibberellic acid (GA_3), indole-3-acetic acid (IAA).

2.8. Respiration measurements

Respiration of flower buds was measured by Warburg's technique on the 2nd, 3rd and 4th day of a 4-day dark treatment and on the 8th day after the treatment, starting from about 12 days before flowering (control plants). Fresh weight was measured immediately and the bud divided into two longitudinal parts, which were inserted into the reaction vessel containing 0.2 ml 10% KOH (w/v) in the centre well and 3.0 ml 0.01 M phosphate buffer (pH 6.6) in the main compartment. The bath temperature was kept at 25°C during measurements.

3. RESULTS

3.1. Effect of illumination on dry weight and carbohydrate content of stem and flower bud

Under low illumination, elongation of the uppermost internode (7th) is strongly suppressed and the flower bud did not develop further. Effects of light treatments on changes in dry weight of stem and flower bud are shown in *fig. 1*. Whereas the dry weight of buds under standard illumination (control plants) increased rapidly, that from plants that, for the period, received weak light or were kept in de dark did not increase at all. Stem growth of the latter two groups was considerably less than that of control plants.

Since HARTSEMA & LUYTEN (1961) and WASSINK & WASSINK-VAN LUMMEL (1953) emphasized the importance of carbohydrates for flower development, the carbohydrate content was estimated nine and two days before flowering. In contrast to the rapid rise in control flower buds, the carbohydrate level in weak

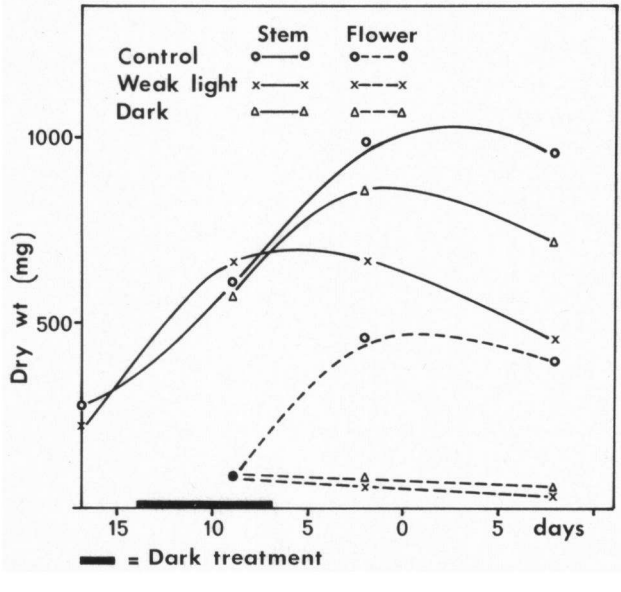

Fig. 1. Changes in dry weight of stem or flower bud during rapid growth of these organs (average of 5 plants). Plants were grown under three different light intensities: control, 56 W m^{-2}; weak light, 56 W m^{-2} until 20 days before flowering, thereafter 11 W m^{-2}; dark from the 14th till the 7th day before flowering began in the control group, before and after dark treatment exposed to 56 W m^{-2}. Day 0 = flowering time.

Table 1. Effect of illumination treatment on carbohydrate content in dry material of stem and flower bud (mg glucose equivalent/g, average of 5 plants). The treatments are the same as in *fig. 1.*

	9 days before flowering	2 days before flowering
Flower bud		
Control	265	297
Weak light	169	73
Dark treatment	237	90
Stem		
Control	362	254
Weak light	265	161
Dark treatment	313	210

light and dark-treated buds strongly decrease during that period (*table 1*). Here it is remarkable that dry weight and total carbohydrate of the stem in control and dark-treated plants did not differ much three days before the treatment stopped.

3.2. Effect of carbon dioxide concentration and light on flowering

One of the important questions arising from the light experiments is: Does light stimulate flower development through the production of photosynthetic products or are other systems involved? To answer this question, different light intensities were combined with normal or low CO_2 concentration. *Table 2* indicates that light, and not CO_2 concentration, is the decisive factor determining the flowering proportion. The fact that normal light gives 100% flowering even if the CO_2 concentration is a tenth of normal indicates that light does not act primarily by synthesizing large enough amounts of photosynthates.

Table 2. Influence of CO_2 concentration and light intensity on dry weight and flowering proportion (proportion of plants in bloom). Treatment starts on the 19th day before flowering (standard condition). Plants were harvested, when control plants flowered.

Light intensity (W m^{-2})	56	2.6	48
CO_2 concentration (% v/v)	0.03	0.03	0.003
Flowering proportion (%)	100	58	100
Dry weight (g)			
Flower	0.30	0.12	0.32
Stem	0.84	0.68	0.62
Leaves	1.32	1.25	1.36
Daughter bulb	0.32	0.30	0.21
Mother bulb	0.93	0.76	0.64
Root	0.38	0.37	0.35
Total	4.09	3.48	3.50

3.3. Distribution pattern of carbon label

In order to know the distribution pattern of ^{14}C label from leaves to other plant parts during rapid stem elongation, $^{14}CO_2$ was supplied to the 2nd or 6th leaf under normal illumination. The distribution of radioactivity was determined 24 h later. Fourteen days before flowering (early stage of stem elongation) the ^{14}C was distributed more or less equally throughout all plant parts. The highest proportion of radioactivity was recovered in the roots (*table 3*).

Table 3. Distribution of radioactivity in various plant parts, 24 h after supplying $^{14}CO_2$ (20 μCi) to the 2nd, or 6th leaf at different days before flowering under standard illumination (each stage one plant). The percentages are relative to total activity taken up by the whole plant, less that remaining in the leaf fed with $^{14}CO_2$.

	14 days before flowering			8 days before flowering			4 days before flowering		
2nd leaf	dry wt. (mg)	μCi	%	dry wt. (mg)	μCi	%	dry wt. (mg)	μCi	%
Leaves	2250	0.34	11.53	2694	0.64	15.38	2090	0.16	5.14
Stem	197	0.46	15.59	901	1.98	47.60	1014	0.47	15.11
Flower	94	0.44	14.92	209	0.68	16.35	469	1.41	45.34
Mother bulb	523	0.53	17.97	611	0.03	0.72	448	0.06	1.93
Daughter bulb	89	0.26	8.80	140	0.35	8.41	109	0.18	5.79
Bulb disk	240	0.23	7.80	356	0.12	2.88	305	0.17	5.47
Roots	1039	0.69	23.39	952	0.36	8.66	832	0.66	21.22
6th leaf									
Leaves	2570	0.19	7.22	3232	0.30	5.83	3016	0.20	4.14
Stem	206	0.36	13.69	904	3.77	73.19	1150	1.74	36.03
Flower	112	0.44	16.73	190	0.89	17.28	614	2.55	52.80
Mother bulb	1280	0.47	17.87	378	0.02	0.39	388	0.02	0.41
Daughter bulb	63	0.34	12.93	102	0.06	1.17	250	0.16	3.31
Bulb disk	321	0.31	11.79	307	0.06	1.17	386	0.07	1.45
Roots	1500	0.52	19.77	659	0.05	0.97	1024	0.09	1.86

Eight and four days before flowering, transport from leaf 6 is mainly directed towards stem and flower. Eight days before flowering, when the stem is actively growing, most activity was found in the stem and 4 days before flowering when the bud was rapidly growing most activity was found in the bud. The second leaf (from base) shows a relatively larger export to bulb and roots, in these two stages.

3.4. Translocation of labelled carbon after the dark treatment

The influence of a preceding dark treatment on translocation pattern of ^{14}C was examined 5 h and 5 days after a dark treatment from the 14th till the 7th day before flowering. Since the data at both stages were similar, only the results of the 5-h experiment are shown in *table 4*. Translocation of the label to a flower bud was hardly observed in the dark-treated plants. The distribution of

Table 4. Distribution of radioactivity in various plant organs, 24 h after supply of $^{14}CO_2$ (20 μCi) to the 2nd leaf (in the light) 5 h after the plant had received a dark treatment, from the 14th till the 7th day before flowering (control plants). The percentage are relative to total activity taken up by the whole plant less that remaining in the leaf fed with $^{14}CO_2$.

	Control			Treatment		
	dry wt. (mg)	μCi	%	dry wt. (mg)	μCi	%
Leaves	2143	0.30	3.7	2163	0.24	18.7
Stem (Internodes 1, 2)	94	0.23	2.8	136	0.19	14.8
(Internodes 3, 4)	238	0.12	1.5	256	0.07	5.5
(Internodes 5, 6)	305	0.18	2.2	271	0.03	2.3
(Internode 7)	165	1.27	15.5	77	0.001	0.1
Flower	517	4.76	58.2	108	0.001	0.1
Mother bulb	453	0.14	1.7	388	0.07	5.5
Daughter bulb	65	0.22	2.7	41	0.18	14.0
Bulb disk	232	0.41	5.0	217	0.25	19.5
Root	1060	0.55	6.7	695	0.25	19.5

radioactivity into internodes shows that translocation of assimilates in the apical direction was suppressed to an extreme degree in the dark-treated plants.

3.5. Translocation of labelled phosphate during dark treatment

To find out the translocation pattern during and after dark treatment, ^{32}P was fed to the 2nd leaf for 24 h on the 3rd and 6th day after the start of the dark treatment (about 14 days before flowering) and 5 days after the dark treatment. Radioactivity was measured 24 h after supply of ^{32}P. No large difference was observed between dark-treated and control plants on the 3rd day; later clear differences were found (*table 5*). The flow of ^{32}P-labelled material into the flower bud stopped almost completely during dark treatment; instead most of the radioactivity was recovered in the stem and bulb of dark-treated plants. Distribution of ^{32}P supplied on the 5th day after dark treatment did not differ

Table 5. Distribution of radioactivity in various plant organs, 24 h after supply of ^{32}P, to the 2nd leaf on the 3rd or 6th day of dark treatment (each stage one plant). The percentages are relative to total activity taken up by the whole plant less that remaining in the leaf fed with ^{32}P.

	Control		Dark treatment	
Day of treatment	3rd	6th	3rd	6th
	%	%	%	%
Leaves	4.1	0.8	8.9	0.0
Flower	14.3	35.5	10.8	0.4
Stem	71.2	32.2	65.1	72.1
Bulb	8.2	16.3	15.2	27.5
Root	2.2	15.2	0.0	0.0

from that obtained after supply on the 6th day of dark treatment (data not reproduced).

3.6. Respiration in the flower bud

Respiration of the flower bud was measured during and after treatment. Relatively small differences were observed between control and dark-treated plants during treatment. On the 8th day (4 days after treatment), a pronounced difference was observed (*table 6*).

Table 6. Effect of a 4-day dark treatment on the respiratory activity of flower buds (expressed as specific rate of uptake of 0_2 in μ litre h^{-1} g fresh wt^{-1}) started about 12 days before flowering. Samples were taken during dark treatment and on the 4th day after the end of dark treatment. Control plants were not in the dark.

	Dark treatment			After dark treatment
Day of sampling	2nd	3th	4th	4th
Treated group	430	386	379	150
Control	529	451	430	392

3.7. Effect of growth substances applied under conditions that led to bud blast

Since it is well known that growth hormones affect flowering of many plant species (AUDUS 1972), an attempt was made to overcome bud blast by injecting growth substances into the flower bud of dark-treated iris plants. Whereas IAA did not have any effect, cytokinins in three replicates, using 14 plants, did partly overcome bud blast. The order of activity was as follows: BAP > zeatin > kinetin > adenine > 2iP; Ga_3 was as active as adenine (*tables 7* and *8*). The optimum concentration for both BAP and zeatin was found to be 10^{-4} M. In comparison to the light-treated controls, the plants flowered on the same day or one to two days later.

4. DISCUSSION

Owing to temperature treatments during dry storage, bulbs could be obtained commercially that produced flowers at any desired period of the year. Apart from this flowering response, these pretreatments may have resulted in slight physiological differences. There may also have been differences between experiments arising from differences in natural illumination before transfer of the plants to the growth chambers. Hence, plants from different experiments may not be strictly comparable, for instance in dry weight.

The results of *fig. 1* and *tables 1* and *3* corroborate the assumption of the occurrence of a critical period (from about the 14th till the 7th day) before flowering, in which light intensity is of clear importance for flowering. These

Table 7. Effect of various growth substances on flowering of iris (14 plants per treatment), injected on the 3rd day of a 7-day dark treatment. Dark treatment started about 19 days before flowering of control plants.

Concentration of hormones	0	10^{-4} M	10^{-6} M
	Flowering proportion (%)		
Control (light)			
Water (injected)	93		
Treatment (dark)			
Water (injected)	0		
BAP		71	7
Kinetin		21	0
Adenine		14	0
2iP		0	0
GA_3		14	0
IAA		0	0

Table 8. Effect of cytokinin concentration on flowering of iris (14 plants per treatment), injected on the 3rd day of a 7-day dark treatment. Dark treatment started about 19 days before flowering of control plants.

	Flowering proportion (%)
Control (light) Water (injected)	100
Treatment (dark) Water (injected)	21
BAP 10^{-3} M	79
10^{-4} M	93
10^{-5} M	43
10^{-6} M	29
Zeatin 10^{-3} M	57
10^{-4} M	64
10^{-5} M	57
10^{-6} M	43

results confirm those of HARTSEMA & LUYTEN (1961) and FORTANIER & VAN ZEVENBERGEN (1973).

Fig. 1 and *tables 1* and *3* also confirm that the light effect is positively correlated with dry weight or carbohydrate content (HARTSEMA & LUYTEN 1961, WASSINK & WASSINK-VAN LUMMEL 1953). The above results could suggest that photosynthetic activity in the sensitive stage is of primary importance for flowering proportion.

Although some CO_2 may be produced by respiration under low CO_2 concentration, the results of *table 2* and the experiments on the distribution of ^{14}C and ^{32}P (*tables 4* and *5*) prove that the influence of light intensity on flowering is not due exclusively to photosynthetic activity; rather, this effect is con-

nected with the distribution pattern of carbohydrates.

Respiration experiments show that decreasing transport of photosynthates to the flower bud in the critical period can result in an aftereffect. Although plants received normal light again, a sharp decrease occurred on the 4th day after the treatment. The relatively small differences during the treatment prove that the flower bud was still functionally active during treatment (*table 6*).

From *tables 2*, *4* and *5* and the observation that the last internode does not elongate in blasted irises, it is concluded that some other factors seem to be involved in bud blast.

Since injection of cytokinins clearly overcomes bud blast symptoms (*tables 7* and *8*), cytokinins seem to be involved in the phenomenon. Probably they play a part in the distribution of carbohydrates. The results agree with the effect of kinetin on the acceleration of flowering (TEPFER et al. 1966).

To test this theory, the influence of growth substances on the distribution of ^{14}C assimilates and the endogenous cytokinin distribution needs to be investigated. Furthermore it would be interesting to analyse the light effect in the critical period of stem elongation and flower bud development in treatments with growth substances.

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