

The control of flowering¹

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Summary

A control of flowering is important in the growing of any crop plant.

The juvenile phase is the period of vegetative growth during which flower formation is impossible. Its duration may vary from a few weeks to several years among different plants. In herbaceous plants the duration of the juvenile phase is modifiable and high light intensity reduces it. During the juvenile phase the necessary substrate for the formation of flowers is lacking.

In "neutral" plants flower formation takes place without the action of specific factors. In several cases an optimal vegetative growth is accompanied by optimal flowering. Thermoperiodicity may play a part, but this factor is not specific.

A prerequisite for vernalization is the presence of dividing cells during the cold treatment. After vernalization a transmissible flowering-stimulus may be formed, but this has been demonstrated in few cases only.

In short-day plants both a transmissible flowering-stimulus and a flowering-inhibition have been established. The inhibition by light is not formed at low temperature or at low light intensity, which makes it possible to induce flowering of short-day plants in long day and even in continuous light.

From a discussion of the mutual relationships between vernalization and photoperiodism, it is concluded that in one and the same plant — *Campanula medium* — at least two different mechanisms of flower formation occur.

Gibberellins cannot be considered to represent a universal flowering hormone. The direct action of gibberellins seems to be stem elongation, followed by flowering in those plants where stem elongation precedes flowering.

The hypothesis of one universal flowering hormone is rejected. The flowering of each plant should be considered as a separate case. A general directive in explaining flowering is that both flowering stimuli and flowering inhibitors may occur, while sufficient substrate must be present.

Additional remarks with respect to control of flowering in practical plant cultivation are as follows. A too rapid flower formation should be avoided. In some cases flowering "the year round" has been achieved. Such a complete control of flowering is important for research purposes, for the growth of valuable horticultural plants and for breeding-projects of all plants.

1. Introduction

With regard to the control of flowering our cultivated plants can be divided into two groups after the plant organ that constitutes the yield:

1. Plants from which a vegetative organ is used: beets; leaf, stem and root vegetables; leaf ornamentals; wood yielding trees; also all plants which have to be

¹ Lecture held at the course "Fundamentals of dry-matter production and distribution" organized by the Royal Netherlands Society for Agricultural Sciences, Wageningen, 10th January, 1962. Also published as: Publication 228, Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool, Wageningen.

propagated vegetatively. On account of the usually existing antagonism between flowering and vegetative growth, no flowering is desired, of course except when these plants are grown for seed or are used in a breeding project.

2. Plants which are grown for their flower (ornamentals), their fruits (fruit crops and fruit vegetables, properly speaking also the cereals), or for their seeds (dry legumes, all seed crops). In this case flowering is a very essential part of the development.

Hence flowering is always important in the cultivation of plants and a negative or a positive control of flowering is an essential part in the growing of most plants.

When the development of a plant starts as a germinating seed the plant passes first through a stage of vegetative growth, from which a juvenile phase can be separated. By definition this juvenile phase is a period during which the formation of flower buds is impossible. This becomes possible during the vegetative adult phase which follows the juvenile phase. Sometimes the flower-bud formation then takes place without a known specific environmental influence, but frequently specific factors like the vernalizing action of low temperature or the influence of daylength can be recognized. Besides the "neutral plants" which flower out of themselves, we distinguish cold sensitive and cold requiring plants, day-length sensitive and day-length requiring plants.

Of course the above facts are generally known and reference to them was only made as an introduction to the following contribution in which I shall select from the multitude of data on control of flowering some items which have been subject of own research. These items are: the juvenile phase; vernalization, photoperiodism and their mutual relationship; gibberellins; a general theory of flowering. I shall end by making some remarks on the control of flowering in plant cultivation. It will be only possible to deal with some aspects of each of these items.

2. The juvenile phase

We already defined the juvenile phase as the period of vegetative growth during which the plant cannot be induced to form flower buds. In first instance we are inclined to suppose that during this period the plant is insensitive to specific flower-bud forming factors, e.g. to the vernalizing action of cold, but we shall have to revise this concept.

The duration of the juvenile phase varies greatly from species to species. The peanut which possesses the primordia of a flower bud in its seed, is a case by itself. Almost always the existence of a juvenile phase can be demonstrated, but it may vary from a few weeks or months in most herbaceous plants to several years in woody plants and — unexpected combination — in most bulb flowers. Immediately the question presents itself whether the duration of juvenility is a fixed character or whether it is subject to modification. In collaboration with HIGAZY (WELLENSIEK and HIGAZY, 1961) it could be demonstrated that the juvenile phase in the biennial *Lunaria biennis* can be strongly influenced by the growth factors during the period from sowing to vernalization. Especially a high light intensity shortens the duration of the juvenile phase considerably and a photosynthetic effect is evident.

Of course this result may not be applied to woody plants and bulb flowers without further research, but it can be considered as a directive. Stress should be laid on the fact that the possibility of carrying out a systematical breeding project with

these plants, characterized by very long juvenility, depends on the possibility of a considerable reduction of the length of the juvenile phase. Several investigators are of the opinion that the juvenile phase in woody plants can be shortened by grafting on weak stocks, but no general agreement is found in the literature. This is doubtlessly caused by the difficulties to devise a conclusive experiment.

Lunaria biennis has also proved to be a good test plant in an attempt to get a better insight into the nature of juvenility. Seed vernalization has no visible effect in this typical biennial. However, when seed vernalization is followed by plant vernalization, the former appears to have exerted an effect all the same, because in this case plant vernalization induces flowering in younger plants, while in addition the percentage of flowering and the rapidity of flower-bud formation are favourably affected (WELLENSIEK, 1958 b). Quite an analogous situation occurs in beets (WELLENSIEK and VERKERK, 1954). It is evident that seed vernalization, hence low temperature in the stage of germinating seed, does have an inductive effect on the flower-bud formation, but that a complete realization of flower-bud formation is impossible through a lack of the necessary building and/or energetic substances, in brief: through a lack of substrate. In recent experiments of ZEEVAART (written personal communication) it was demonstrated that during the juvenile phase of *Bryophyllum* no flowering stimulus is formed. In this plant the juvenile phase lasts very long, about one year, but ZEEVAART could induce flowering in very young seedlings by grafting them on flowering plants. This result is in favour of the idea that juvenile plants do not flower on account of a lack of substrate, in this case a lack of substrate to form the flowering stimulus.

3. "Neutral plants"

Before passing to a discussion of the specific action of cold and daylength, a few remarks should be made on the group of plants which already has been indicated as "neutral". No specific factor is known which induces them to flower. In theoretical discussions on flowering this group belongs to the underdeveloped subjects. In discussions on the cultivation of neutral plants, our lack of knowledge is demonstrated when *f.i.* we should attempt to prevent flowering in typical representatives of this group, *e.g.* most garden beans. Perhaps this is only possible by creating an environment which completely suppresses vegetative growth, in other words which kills the plants. Possibly a general rule is that optimal vegetative growth is accompanied by optimal flowering. However, there are exceptions, because sometimes optimal flowering takes place when nitrogen is minimal.

The tomato is a good case of a plant in which control of flowering is possible by regulating the light intensity and the temperature. Diurnal thermoperiodicity appears to be an important factor, but it is certainly not specific. In bulb flowers the well known investigations of BLAAUW, VAN SLOGTEREN and collaborators have led to control of flowering through regulation of the temperature to such an extent that flowering the year round has become possible. Again, thermoperiodicity plays a substantial part, in this case not diurnally, but during longer periods. No more than in the tomato, this factor can be considered as specific.

4. Vernalization

Vernalization is a specific factor in the flowering of the cold-requiring plants, be-

cause flower-bud formation occurs after a cold treatment only. It is generally known that in certain plants seed vernalization is effective (winter annuals), while in other plants vernalization is only effective when applied to adult plants (plant vernalization of the biennials). Both forms of vernalization have already been mentioned before.

It is generally accepted that the only locus of vernalization is a growing point, subsidiary a bud. Recent investigations have shown that this concept cannot be maintained and that leaves can also be vernalized (WELLENSIEK, 1961 a, b). Again, *Lunaria biennis* has served as experimental plant, because leaf cuttings *without buds* can be brought to adventitious root formation quite easily and to adventitious shoot formation rather easily. This makes it possible to vernalize leaf cuttings without buds, to make them regenerate after the cold treatment and to observe whether flowering will occur. This has indeed taken place, but the leaf cuttings must be taken from adult plants and the cold treatment must last rather long. As a side-result, the regeneration as such appeared to take place much better in leaf cuttings from juvenile plants, while it was influenced favourably by the preceding low temperature treatment.

Also root vernalization was possible. Doubtlessly this discovery of leaf and root vernalization will influence our insight into the nature of the vernalization process¹.

Anyhow, *Lunaria biennis* has been quite a useful experimental plant and this holds true also for the demonstration of a transportable flowering stimulus which is formed in the plant after vernalization. When shoots of flowering plants are grafted on vegetative adult stock plants, buds from the stock may give rise to shoots which flower completely. This transmission of a flowering stimulus occurred in about 70 % of the successful grafts. Together with the case of *Dianthus barbatus* as studied by WATERSCHOOT, this is one of the very few demonstrations of a transportable flowering stimulus after vernalization. Recent results of CHOUARD and collaborators (CHOUARD, 1962; HARADA, 1962) indicate that after vernalization the amount of gibberellins has increased. If indeed the explanation of vernalization would be the formation of gibberellins, the supposed flowering stimulus would be a gibberellin, and the question presents itself whether vernalization should not be explained via an influence on stem elongation. I shall discuss this point later. For the rest it is difficult not to see vernalization as the disappearance of a flowering inhibition. Perhaps both concepts hold true. This is certain with regard to our next item: photoperiodism.

5. Photoperiodism

Of course the existence of day-neutral, long-day, short-day plants is generally known. Less known are the long-short-day plants which for flower induction need long day first, but short day afterwards. Still less known are short-long-day plants, with *Campanula medium* as a typical representative which will be discussed later. These types of reaction have been mentioned, because thusfar numerous attempts to unite them under one theoretical viewpoint have failed, so that each should be considered as a separate type.

Especially short-day plants are interesting, because they show quite convincingly both flowering stimuli and flowering inhibitors. In addition, the first effect of short day

¹ When preparing the manuscript for the press: New results have made it clear that the locus of vernalization is to be found in cells which are dividing (Wellensiek, 1962).

in *Perilla crispa* according to ZEEVAART (1958) is an "induced state" in the leaves which is not transportable and hence evidently is a condition of the protoplasm. Leaves in the induced state produce a flowering stimulus and this is transportable. In *Perilla crispa* (WELLENSIEK, 1958 a, 1959), but quantitatively much stronger in *Salvia occidentalis* (WELLENSIEK, 1960 a), the existence could be demonstrated of a flowering inhibition which is induced by (too much) light and disappears by (long enough) darkness. This light inhibition explains why short-day plants do not flower in long day: the nights are too short to remove the light inhibition. This inhibition was demonstrated by periodically interrupting the short-day treatment with 2 or 3 days of continuous light. It appeared that only the origin of the induced state is inhibited and not the production of the flowering stimulus. The light inhibition does not arise at low temperature and neither at very low light intensity. This makes it possible to induce flowering of short-day plants in long day and even in continuous light.

6. The mutual relationship between vernalization and photoperiodism

An undeniable mutual relationship exists between vernalization and photoperiodism. Thus, without exception, seed vernalization leads to a complete effect only when followed by long day. This does not hold true for plant vernalization which frequently is accompanied by day neutrality, sometimes by long-day requirement and even, although very rarely, by short-day requirement.

According to the well known work of GREGORY and PURVIS the action of the low temperature in winter rye can almost completely be replaced by short day. This is certainly not generally true, however. The opposite also occurs. In our laboratory BARENDSE (unpublished) found that in *Cheiranthus allionii* a short-day treatment of limited duration already inhibits the effect of vernalization, while a longer short-day treatment has a completely devernalizing action.

Campanula medium is quite a good plant to illustrate the mutual relation between vernalization and photoperiodism, because this plant can be brought to flower both by plant vernalization, (usually) followed by long day, and by short day, (always) followed by long day (WELLENSIEK, 1960 b). It is tempting to suppose that in this case vernalizing cold and short day have the same action. Short day would then actually replace cold, and the reverse. However, it has turned out that this hypothesis fails completely. Quite a number of observations demonstrate that the mechanisms of vernalization and of short-day action must be completely different. To mention just two:

- a. the juvenile phase for vernalization lasts decidedly longer than for short day;
- b. during a permanent cold treatment a slow stem elongation and a beginning of flower-bud formation occur, but during a permanent short-day treatment the plants remain in rosette form and remain completely vegetative. We conclude to the existence of two very different mechanisms of flower-bud formation *within one and the same plant!*¹

¹ When preparing the manuscript for the press: In recent experiments it was found that 4 weeks of cold followed by 4 weeks of short day have a completely different effect than 4 weeks of short day followed by 4 weeks of cold. The latter treatment gives much more and much faster flowering.

7. Gibberellins

Since ANTON LANG (1956) demonstrated that biennial *Hyoscyamus niger* is induced to flower without vernalization, but after treatment with gibberellin (GA), and since naturally this led to the idea that GA could be the long sought flowering hormone, a discussion of gibberellins cannot be avoided in a review on control of flowering. I shall try to formulate briefly some general conclusions from the mass of literature. We then see that GA:

1. has the same effect as cold in some plants, but not in others;
2. has the same effect as long day in some plants, but not in others;
3. has never the same effect as cold + long day;
4. has never the same effect as short day, perhaps with a single still uncertain exception.

From these conclusions follows with certainty that GA very decidedly is not *the* universal flowering hormone. However, some reserve is needed, because a great difference of action between different gibberellins may occur. As an illustration, ANTON LANG and JAN ZEEVAART (in the press, written personal communication) found that GA₇ has by far the strongest action, also in some cases where GA₃ does not exert any action at all.

In an attempt to analyse the action of GA somewhat further, it appears that in most cases, if not always, the direct action of GA is stem elongation, followed by flower-bud formation as a direct effect of this stem elongation, but as an indirect effect of GA. From this it would follow that GA can influence only the flower-bud formation of those plants, where stem elongation precedes flower-bud formation. This hypothesis still needs a systematical test.

8. A general theory of flowering

From time to time in the foregoing I already discussed some theoretical viewpoints. I shall now try to round them off by discussing the question whether one general theory of flowering is feasible. The first attempt, the theory of KLEBS, has already become history and is too often forgotten. Especially many practical measures in fruit growing are based on KLEBS and are effective. From a historical standpoint it is interesting that KLEBS has come close to the discovery of both vernalization and photoperiodism. When these phenomena had become generally known and when the existence of a transportable flowering stimulus was demonstrated in grafting experiments, of course the hypothesis was pronounced that there would be one universal flowering hormone, responsible for the flower-bud formation in all plants. It is striking how gratuitously and uncritically this hypothesis has been accepted and how easily terms as "florigen" or "anthocaline" or "anthesine" came into use without the possibility of attaching to them something concrete. It is clear that I am not an advocate of the theory of the universal flowering hormone and I mention three arguments for this negative attitude:

1. Notwithstanding numerous attempts, the isolation of the flowering hormone with biochemical methods has not succeeded. However, it may be that LINCOLN *et al.* (1961) have recently isolated a specific substance which determines flower-bud formation in *Xanthium*. A more universal action has not been studied yet.

2. The identity of flowering stimuli from different plants can never be demonstrated by the method of grafting, because this has its natural limitations due to whether or not the tissues of donor and receptor unite after grafting.
3. The strongest argument, more positive than the foregoing, starts from the existence of at least two different mechanisms of flower-bud formation in *Campanula medium*, as discussed above. Considering more plants, we find a great number of very different mechanisms which all result in flowering. It seems to be rather improbable that one and the same substance, i.e. the flowering hormone, could be synthesized in so many different ways.

The great counterpart of the theory of the universal flowering hormone is the theory of the flowering inhibition of VON DENFFER (1950). According to this concept every plant would have a natural tendency to flower, but inhibitions may prevent it from actual flowering. These inhibitions may be very different from plant to plant and the action of specific flower-inducing factors would consist of a disappearance of these inhibitions. VON DENFFER founded his theory quite well, but his supposition that auxins are the most important, if not the only inhibitors seems to be untenable.

In the foregoing I have demonstrated some cases of both flowering stimuli and flowering inhibitors and I have done this on purpose with regard to the present discussion. The transportable flowering stimuli can certainly be considered as hormones which act as limiting factors in the formation of flower buds. On the contrary, both the induced state and the light inhibition do not possess a hormonal nature. This does not imply that hormonal flowering inhibitors would not occur; the results of certain grafting experiments point to their existence.

Arriving at a final conclusion, one universal theory of flowering is not conceivable, but every case should be considered by itself. A general directive is that the formation of flower buds can be influenced both by flowering stimuli and by flowering inhibitors, while of course a prerequisite is that sufficient substrate as building material and respiratory substances is available. The future development of the research of flowering is a more detailed analysis of the nature of the flowering stimuli and the flowering inhibitors.

9. The control of flowering in the cultivation of plants

The foregoing discussion already indicated several potential applications. Therefore, the present discussion can be restricted to some additional remarks.

1. In applying a control of flowering, every species and often every cultivar should be considered as a separate case. Of course certain specific types of reaction occur, like the winter cereals, the biennials, short-day plants, long-day plants, fruit trees, but great differences may occur between the representatives of these groups and even between their cultivars. *E.g.*, in rice a whole series of critical daylengths occurs.
2. In the introduction I have already drawn attention to the fact that control of flowering sometimes means promotion of flowering, sometimes suppression of flowering, depending on the plant organ(s) which compose(s) the yield. An instance of suppression of flowering is anti-vernalization: the avoiding of vernalizing low temperatures.

3. In promotion of flowering a too rapid flower induction should be avoided, since this may result in a depression of the productivity.
4. In by far the greater number of plants control of flowering to a certain extent is possible in some or other way. In extreme cases the effect of the natural seasons may be completely overcome and the plants are harvested the year round: bulb flowers, chrysanthemums, lilacs, strawberries. This possibility is also very attractive for research projects.

From a technical standpoint, the natural low winter temperatures may be used for vernalization by regulating the time of sowing. Artificial seed vernalization can be applied in simple kitchen frigidaires. Artificial plant vernalization needs a much more expensive installation and this explains perhaps why relatively little research is done in this field.

An increase of the daylength for promotion of flowering in long-day plants and for suppression of flowering in short-day plants can easily be realized by a combination of incandescent lamps and a switch clock. Decrease of the daylength is realized with more difficulty, but also in this case there are several possibilities: covering structures with matings or cloth, curtains, while also completely mechanized installations occur, but these are expensive.

5. From an economical standpoint, control of flowering in the cultivation of plants is attractive in first instance for valuable horticultural crops. Control of flowering is important for all seed crops, when a rapid increase of small lots is desired. Finally, in almost all breeding projects a promotion of flowering has a great value for shortening the breeding cycle and, hence, make a rapid progress. Suppression of flowering is important, when as part of a breeding program vegetative propagation is applied.

The foregoing discussion could not be more than a rather superficial introduction and several interesting items had to be omitted. However, I hope to have succeeded in pointing out the importance of control of flowering in plant cultivation, but also in demonstrating that control of flowering offers an extremely fascinating subject to the research worker.

LITERATURE

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| CHOUARD, P. | 1962 Rôles respectifs de la gibbérelline et du froid dans la vernalisation. Symposium Giessen 1960. Springer Verlag, Berlin-Göttingen-Heidelberg, 50—60. |
| DENFFER, P. VON | 1950 Blühhormon oder Blühhemmung? Neue Gesichtspunkte zur Physiologie der Blütenbildung. <i>Naturwiss.</i> 37, 296—301, 317—321. |
| HARADA, H. | 1962 Endogenous gibberellins and flowering. Symposium Giessen 1960. Springer Verlag, Berlin-Göttingen-Heidelberg, 45—50. |
| LANG, ANTON | 1956 Stem elongation in a rosette plant, induced by gibberellic acid. Induction of flower formation in biennial <i>Hyoscyamus</i> by treatment with gibberellin. <i>Naturwiss.</i> 43, 257—258, 284—285. |
| LINCOLN, RICHARD G.,
DARWIN L. MAYFIELD,
and ALAN CUNNINGHAM | 1961 Preparation of a floral initiating extract from <i>Xanthium</i> . <i>Science</i> . 133, 756. |
| WELLENSIEK, S. J. | 1958a Photoperiodical reactions of <i>Perilla crispata</i> . <i>Proc. Kon. Ned. Akad. Wetensch.</i> C 61, 552—560. |

- WELLENSIEK, S. J. 1958b Vernalization and age in *Lunaria biennis*. *Proc. Kon. Ned. Akad. Wetensch. C 61*, 561—571.
- 1959 The inhibitory action of light on the floral induction of *Perilla crispa*. *Proc. Kon. Ned. Akad. Wetensch. C 62*, 195—203.
- 1960a Les réactions photopériodiques des plantes de journée courte. *C.R. Acad. Agric. France. 46*, 607—611.
- 1960b Flower formation in *Campanula medium*. *Meded. Landb. hogesch. 60 (7)*, 1—18.
- 1961a Regeneratie en bloei-inductie bij geïsoleerde bladeren. *Versl. Kon. Ned. Akad. Wetensch. 70*, 93—97.
- 1961b Leaf vernalization. *Nature. 192*, 1097—1098.
- 1962 Dividing cells as the locus for vernalization. *Nature. 193* (in the press).
- , and M. K. HIGAZY 1961 The juvenile phase for flowering in *Lunaria biennis*. *Proc. Kon. Ned. Akad. Wetensch. C 64*, 458—463.
- , and K. VERKERK 1954 Annual seed growing of beets. *Neth. J. agric. Sci. 2*, 98—104.
- ZEEVAART, J. A. D. 1958 Flower formation as studied by grafting. *Meded. Landb. hogesch. 58 (3)*, 1—88.