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Organ Correlations Affecting Flowering in Relation to Phytohormones

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Abstract. The influence of different organs on flowering of photoperiod- or temperature-dependent plants and of neutral plants is described. Special attention is given to the influence of roots which generally inhibit flowering. High or low temperatures applied to the roots of *Chenopodium polyspermum*, a quantitative short day plant, induce flowering. Vernalizing treatment of roots of *Cichorium intybus* suppresses the inhibition of flowering arising from the tissues immediately underlying the terminal bud. Other plants such as coniferous trees can flower more intensively if the growth of their roots is reduced. After a short presentation of the very complex situation concerning the regulation of flowering *via* the presently known hormones, the methodological limitations of our understanding of the precise hormone mechanism of flowering will be discussed. One example of an immunologica l approach to localize endogenous hormones inside tissues is given, using abscisic acid.

The influence of different organs on flowering has been shown in diverse situations and in different plants. We can, quite arbitrarily, distinguish two main cases: 1) an organ influences the development of the apex only if it undergoes a physiological change, for instance, after perception of photoperiodic induction; 2) an organ influences apex development *per se*. The first example of flowering regulation includes a primary environmental effect while the second is limited to correlative influences which participate in the necessary coordinative phenomena which control plant development. Whatever the kind of correlative phenomena, it is obvious that hormones have an important place in the mechanisms involved (ZEEVAART 1978). Flowering is largely concerned with this type of regulation of development (MIGINIAC 1978, KREKULE 1979). However, the best known examples of flowering regulation have come from the group of plants in which flowering is directly dependent on environmental factors. Nevertheless, a large number of plants are not or only slightly environment-dependent for their flowering, including many perennial plants. Even photoperiodic plants show clear interactions between the induction process and correlative phenomena. Grafting experiments lead to success which implies the transmission of a floral stimulus usually after more or less extensive defoliation of the non-induced leaves of the receptor plant (ALLOT-DERONNE 1983). The underlying mechanisms of these inhibitory effects originating in non-induced leaves or in roots (MIGINIAC 1978) have not been clearly explained, even if indirect

evidence suggests hormonal effects. It is the same situation for stimulatory effects usually coming from photoperiodically induced leaves.

Therefore, in this article we shall examine some new results which strengthen the idea of correlative influences which inhibit flowering. The difficulties in understanding the hormonal regulation of flowering will be concisely sketched. Finally, suggestions for a new methodological approach will be made.

The Importance of Inhibitory Correlative Effects

In *Scrofularia arguta*, a quantitative long day plant, and in *Chenopodium polyspermum*, a quantitative short day plant, we have shown (JACQUES and MIGINIAC 1985, MIGINIAC 1985) that isolated buds cultured *in vitro* flower very rapidly even in non-inductive conditions, provided the explants do not bear any roots. Similar results have been obtained with *Perilla ocimoides*, a qualitative short day plant (ALLOT-DERONNE 1983). These data show that the apex included in these buds is able to flower without any induction. They also show that organs such as roots can inhibit this endogenous ability to flower. This is a typical example of an inhibitory correlative effect which in the case of the three examples cited can be counteracted by the suitable photoperiodic induction. Therefore it appears that flowering is the consequence of complex interactions between correlative factors, *i.e.* inhibition by roots and stimulation by induced leaves. Such a conclusion can be clearly drawn from the experiments of SHINOZAKI and TAKIMOTO (1983) on *Pharbitis nil*. The suppression of root elongation by different chemical means leads to the long day flowering of this qualitative short day plant.

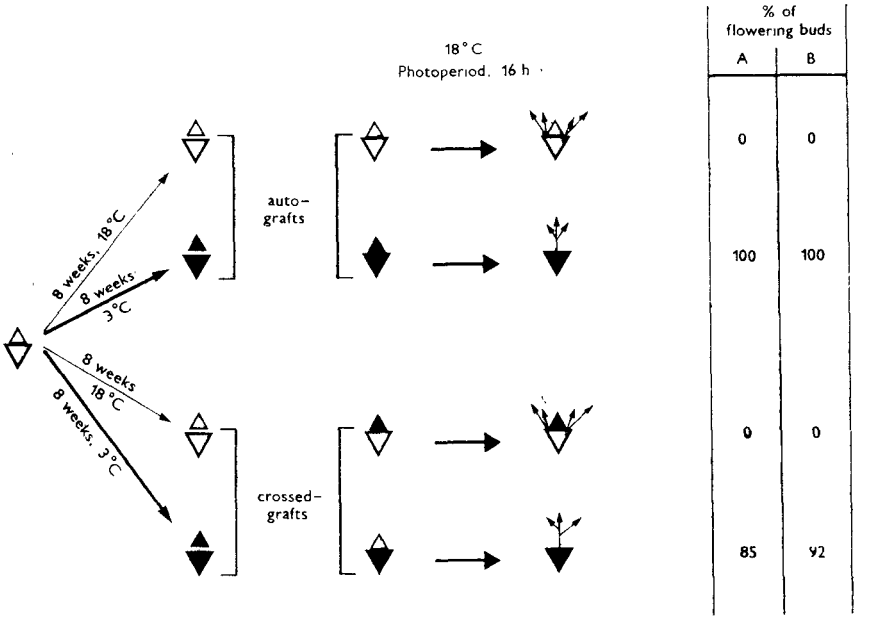

Fig. 1. Behaviour of apical bud of *Cichorium intybus* grafted on to its original root or on to another root treated or not by cold temperature. — Black: 3 °C treatment; white: 18 °C treatment. — A: early cultivar. B: late cultivar (after JOSEPH 1984).

TABLE 1

Influence of the length [cm] of the root underlying tissue on the % of inflorescences formed by isolated buds of *Cichorium intybus* (after JOSEPH 1984)

N° of weeks at 3 °C	Length of the root underlying tissue [cm]	% of flowering buds
0	0	83
	1	60
	2	11
8	0	87
	1	88
	2	87

Even when the plant is a qualitative photoperiodic one and, in contrast to *Perilla ocimoides*, cannot flower without a suitable inductive treatment, it is possible to show a typical inhibitory effect of the roots. This is the case with the long day plant *Anagallis arvensis*, where a clear correlation between rhizogenesis in cuttings and inhibition of flowering is observed (BISMUTH *et al.* 1979). This correlative effect is maximal when the root stock per cutting has been formed.

A recent work has shown that interaction between correlative actions involving again roots and buds and cold treatments could play an important role in the flowering of *Cichorium intybus* (JOSEPH 1984). This plant needs cold treatment before being induced to flower by long days. The results of the diverse grafting experiments summarized in Fig. 1 clearly demonstrate that non-vernalized roots inhibit the flowering of the terminal bud. A non-vernalized bud can form flowers only if it is connected with root tissue where transformations induced by cold treatment have reduced or suppressed the flowering inhibitory effect that this organ exerted on the bud (Table 1). As in *Scrofularia arguta* and *Chenopodium polyspermum* (MIGINIAC 1978) isolated buds not previously submitted to an inductive treatment can form inflorescences on condition that the volume of the underlying tissue is very small (Table 1). These results show that buds do not need any stimulation to change their morphogenetic pattern. In fact, the shift from vegetative development to reproductive development is the consequence of the suppression of inhibitory correlative actions. Isolating the buds from the roots is the means to get early flowering. Another way is to submit the whole tissue (root and bud) to an external factor which acts not on the meristem cells but on the root tissues, weakening or suppressing the inhibitory effect. These recent results fit in very well with suggestions of CHOUARD and TRAN THANH VAN (1970) about the mode of action of vernalization treatment in *Geum urbanum*.

If, to avoid secondary effects of injuries done to tissues at the moment of excision of an organ to break a given correlation, one modifies the physiology of the donor organ without wounding it, using for instance temperature treatments, one can obtain results similar to those given by organ suppression experiments. An example is a work done with *Chenopodium polyspermum* (CHAMONT *et al.* 1982), where treatment of the roots with hot (37 °C, Fig. 2)

or cold temperatures (7 °C, Fig. 3), while the aerial organs stay at 22 °C, considerably reduces the growth of these organs and significantly stimulates flowering. These temperature treatments create water stress in the plants (CHAMONT *et al.* 1982), which could be related to the flowering as pointed out by BERNIER *et al.* (1981), although this aspect of flowering physiology has not been extensively explored.

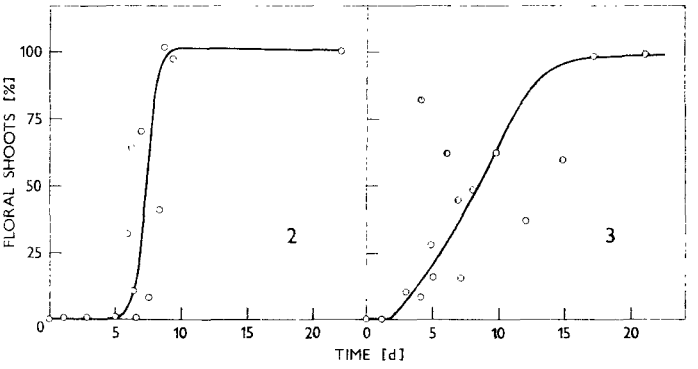

Fig. 2. Influence of the duration of 37 °C treatment given to the roots of *Chenopodium polyspermum* in long days (non inductive photoperiod). The aerial organs are at 22 °C. Observations made on 16 shoots 28 days after the beginning of the experiment (after CHAMONT 1982).
Fig. 3. Influence of the duration of 7 °C treatment given to the roots of *Chenopodium polyspermum*. The aerial organs are at 22 °C. One long night insufficient to induce control plants was given at the beginning of the thermic treatment. Observations made on 16 shoots 28 days after the beginning of the treatment (after CHAMONT 1982).

If correlative phenomena have any significance in the flowering physiology it is in connection with flowering of perennial plants and especially of trees. ROMBERGER and GREGORY (1974) observed that trees survive because their flowering is not controlled directly by photoperiod as it is in many herbaceous plants. As we have seen that organ correlations are involved in the flowering of herbaceous plants even when this phenomenon is environment dependent,

TABLE 2
Species sensitive to gibberellin inducing flowering treatments (after BONNET-MASIMBERT 1983)

Gibberellins	Species
GA_3	*Cupressus arizonica; C. sempervirens* *Chamaecyparis* spp. *Thuja plicata*
$GA_{4/7}$	*Pseudotsuga menziesii* *Larix decidua; L. leptolepis* *Pinus contorta; P. taeda; P. radiata; P. sylvestris* *P. banksiana; P. elliotii; P. palustris* *Picea sitchensis* *Tsuga heterophylla*

one should find such examples with trees. It is the case, for instance, with *Pseudotsuga menziesii*, where different experimental means used by BONNET-MASIMBERT (1982) clearly indicate that only plants with no root growth during the treatment produce flowers. As in numerous other coniferous plants, gibberellins stimulate flowering in young *Pseudotsuga menziesii* (Table 2).

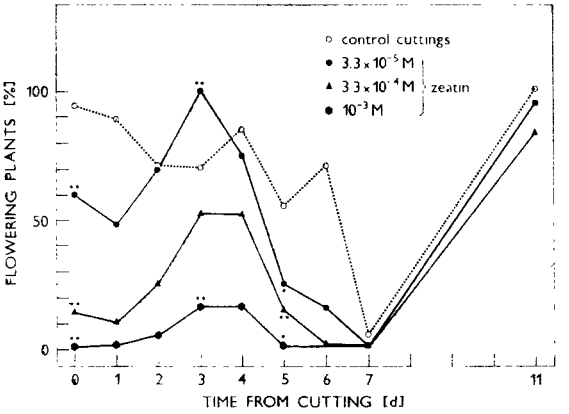

Fig. 4. Influence of zeatin on flowering in *Anagallis arvensis* cuttings during root development. Experimental cuttings were grown at 22 °C in short day (9h-photoperiod) and submitted at different developmental stages (0 to 11 days) to a single inductive long day (24h-photoperiod). The cuttings were then transferred to short days. Zeatin treatments were made during the inductive period. Control cuttings were similarly treated by water. Differences from control are significative at 95 % (.) and 99 % (..) confidence level. (After BISMUTH and MIGINIAC 1984, modified).

But, root hypoxia, a very efficient treatment for stopping root growth, stimulates male and female flowering in the absence of an exogenous gibberellin supply to the trees.

We can conclude with ROMBERGER and GREGORY (1974) that there are "controlling systems whereby external factors that favour flowering (such as the photoperiod at some time each year) are counteracted by internal factors that prevent it". We have tried to show that roots are included in these internal factors.

The Role of Phytohormones in Flowering. How to Study the Hormonology of Flowering?

Although all correlative effects cannot be explained in terms of hormones, there are a considerable number of results which show that these substances are involved in the flowering process.

Even if we limit the discussion to the induction and evocation phases, we must agree with the conclusions of the chapter of the book written by BERNIER *et al.* (1981), which is devoted to this question. No general principles can be stated, although many results have been obtained. Probably, the gibberellins represent the class of phytohormones most involved in the flowering process, even when they are unable to provoke flowering when applied to plants. Other factors interact with these hormones. In coniferous plants, about 15 species react to gibberellin treatments. According to BONNET-MASIMBERT (1983), GA_3 and $GA_{4/7}$ are the main forms to be active (Table 2).

We could present arguments in favor of the influence of one or other class of phytohormones on flowering. It would soon appear, as clearly shown by BERNIER *et al.* (1981), that if one substance is active in promoting or inhibiting flowering, the context of its action is very important: species, physiological state, other phytohormones, nutrition, represent factors which can interact with any hormonal treatment. An example of this is given by the recent work of BISMUTH and MIGINIAC (1984), who have shown that zeatin treatment of cuttings of *Anagallis arvensis* that are forming roots gives different results according to the kinetics of root formation (Fig. 4). Usually, zeatin inhibits flowering, but when the initiation of root primordia begins inside the cutting tissue (day 3), the amount of zeatin which inhibited flowering one and two days before becomes able to stimulate it slightly but significantly.

In fact, our knowledge of the endogenous hormonal status of a plant during transition to flowering is very poor. It appears to many specialists that the regulation of plant morphogenesis is plurifactorial. This point of view has been largely presented by BERNIER (1976), MIGINIAC (1978) and KREKULE (1979) for flowering. In this plurifactorial conception, hormones play an important role. But our knowledge comes essentially from indirect observations, such as suppression of correlations, hormonal treatments, study of the penetration, transport and metabolism of a radio-labelled hormone. Although interesting, these observations do not allow us to establish a strict causal relation between the amount of one compound and induction of a given morphogenesis, in the context of the many factors determining it. The reasons for this are essentially methodological: to measure with certainty a hormonal compound is indeed a quite difficult task which needs the use of physicochemical methods like HPLC and GC-MS (BRENNER 1981). *A fortiori*, the setting up of the hormonal balance is much more complex. Even if one were able to get sufficient data to list the quantitative evolution of the known hormones in the apex tissues during the phases of evocation and morphogenesis, an important aspect would escape the analysis. This is the structural and functional heterogeneity of the meristematic tissues which cannot be appreciated from analytical methods which destroy the structure. Therefore to obtain the maximum amount of quantitative data and to describe the functional evolution of the sites of morphogenesis without destroying them, we thought that only immunological methods could be successful.

WEILER (1982) has discussed the advantages of immunological methods to obtain data quickly on the amounts of the main hormones in small volumes of tissue. To our knowledge, only one paper (ZAVALA and BRANDON 1983) has described an immunocytochemical method of localization of phytohormones. It shows a root localization of cytokinins using an anti-dihydrozeatin serum. Our own assays began with an anti-abscisic acid serum and the material used was *Chenopodium polyspermum*, submitted or not submitted to water stress. The reasons for this approach were two-fold. Firstly, it is well known that water stress provokes an increase of endogenous ABA. We have verified this point with a very useful physiological test to validate the histological observations. Secondly, we know from the work of CHAMONT *et al.* (1982) that water stress occurs when roots are submitted to temperature treatments which were able to provoke flowering. In addition, ABA could be involved in flowering of *Chenopodium polyspermum* as it was in *Perilla crispa* (PURSE 1984). The main steps of the method used to localize ABA in

Fixation procedure:

- Fixation under vacuum of endogenous ABA onto proteins with EDC (soluble carbodiimide) by immersion of the samples in a 2 % aqueous solution.
- Post-fixation in a 4 % paraformaldehyde + 1 % glutaraldehyde mixture in phosphate buffer, pH 7.2.
- Dehydration in ethanol before embedding in spurr epoxy resin or in *n*-butanol or in ethanol before embedding in paraplast.

Confection of sections:

- Confection of sections which were affixed on glass slides precoated with gelatin (0.5 %) and dried overnight at 50 °C.
- Before immunological treatment, paraplast sections were dewaxed with toluene and graded ethanol.

Immunological staining. All treatments were carried out at room temperature. The main steps are:

- Application of drops of IgG anti-ABA on the sections overnight, in darkness and in moist chamber.
- rinsing in PBS. T (phosphate buffer saline + 0.1 % Triton × 100)
- incubation with goat anti-rabbit IgG
- rinsing in PBS. T
- staining by the PAP (peroxidase anti peroxidase) complex (STERNBERGER *et al.* 1970).

Fig. 5. Protocol for immuno-histological localization of hormone. Example of ABA in *Chenopodium polyspermum*: main steps of the protocol.
Controls have to be done to confirm the specificity of the first antibody (incubation made with anti-ABA IgG pre-absorbed for 24 h at 4 °C with an excess of ABA) and to test the specificity of the label (omission of each reagent tested one at a time; substitution of the primary anti-serum by non immune rabbit or pre-immune IgG in dilutions matching those of the primary anti-serum; use of increasing dilutions of the primary anti-serum).

different organs of *Chenopodium polyspermum* are summarized in Fig. 5 and explained in SOTTA *et al.* (1985). The observations are much more of methodological usefulness than biological interest. They show essentially that if sufficient care is taken (numerous controls to test the efficiency of each step of the methodology and particularly the specificity of label) it is possible to draw out a general scheme showing the localization of endogenous ABA. Its repartition is very heterogeneous. The staining is much denser in inhibited axillary buds than in the active terminal bud. Water stress enhances the staining in the same organs as it does in the chloroplasts of leaves.

These observations show only the possibilities of the immunological techniques. We think that, in the near future, using anti-sera raised against different hormones, we will be able to describe more precisely the evolution of endogenous hormones in relation to the first steps of the floral process. We shall try firstly to determine if modifications of the distribution of hormones inside the meristem can be correlated with floral evocation processes. Quantitative data have to be obtained simultaneously because the histo-

logical observations cannot be quantified. Another question to be studied will bear on the eventual hormonal mechanisms of flowering inhibition as discussed above.

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BOOK REVIEW

LEMON, E. R. (ed.): CO_2 AND PLANTS. The Responses of Plants to Rising Levels of Atmospheric Carbon Dioxide. AAAS Selected Symposium 84. — Westview Press, Inc., Boulder 1983. 280 pp. US $ 32.50.

To predict how higher levels of CO_2 may affect global climate, photosynthesizing plants, and thus agriculture and the whole biosphere becomes nowadays increasingly important. The biosphere as well as lithosphere, hydrosphere, and atmosphere of the Earth are in dynamic equilibrium. It is now understood that atmospheric carbon dioxide concentrations due to human activities increase every year and that the acceptable levels of CO_2 should not be more than 850 cm m^{-3}, *i.e.* no more than 1.5 to 2.5 times the natural CO_2 concentration.

This volume arose out of the Workshop held at Annapolis, MD, April 2—6, 1979. The topic was the Environmental and Societal Consequences of a Rising Level of Atmospheric CO_2. Following the Annapolis Workshop a meeting was held in Athens, Georgia, May 23—28, 1982, under the title "Rising Atmospheric Carbon Dioxide and Plant Productivity: An International Conference". The reviewed book contains the proceedings of this meeting. It includes eight chapters compiled by the chairmen and the co-chairmen of the respective panels. The book focusses on the influence of higher level of CO_2 on carbon metabolism of plants, on photosynthetic rate and photosynthetic response of representative C_3, C_4 and CAM plants, on plant growth and development, soil microbial activity, nitrogen fixation and mycorrhizal activities, on nitrogen-fixing, nitrifying and denitrifying bacteria, mycorrhizal fungi and plant pathogens. Responses of terrestrial plant communities, as well as aquatic plants to elevated concentrations of atmospheric CO_2 are also included. Interpretive Summary was placed at the beginning of the book and Sumnary and Recommendations section at the head of each chapter, which allows the reader to grasp the essence of the chapter at the start. This arrangement allowed the editor to unify a large amount of information and experience of a number of contributors. The only disadvantage is the absence of any index. In spite of that, the result is an excellent manual welcome by plant scientists engaged directly or indirectly in the studies of plant growth and functioning in increased CO_2 concentrations.

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