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The Role of Auxin in Inductive Phenomena

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Abstract. The current status of our knowledge of auxin effects on floral induction is summarized. The most general effect is inhibition, although the concentration of synthetic auxins added to plants tends to be too high for us to be certain that the inhibitory effects are truly physiological. Studies of endogenous levels of auxin have focused almost entirely on IAA-like bioassay activity. Chemical identifications of endogenous IAA are needed and feasible. In addition, a search for other auxins involved in vegetative to floral transitions, their chemical identification, and measurement of their changing levels in the plant are urgently needed.

I have been asked by the organizers of this Symposium to review briefly the current status of research on auxin in the induction of flowering and to suggest possible future avenues for exploration.

It is highly appropriate that this meeting should be held in Czechoslovakia because the first report that auxin could function as a floral inhibitor was that by DOSTÁL and HOŠEK in 1937. In addition, since 1937 the effect of added indol-3-ylacetic acid (IAA) and other auxins on flowering has been quite thoroughly investigated, with the timing and location of such effects being a special interest of the Institute of Experimental Botany of the Czechoslovak Academy of Sciences.

In the late 1930's and 1940's the failure of scientists to find the hypothesized single flower stimulating hormone drove them to look for other explanations. The idea that auxins were flower-inhibiting substances and that the induction of flowering might involve anti-auxin effects became popular. The easiest path to follow was to add auxin to plants; many such publications appeared. I remember the first such report at national meetings in the USA: a large dose of IAA was reported to inhibit flowering. Solon Gordon asked in his usual polite way if there was any evidence that this large dose stopped all development, thus making the relation with flowering completely nonspecific. The speaker rather shamefacedly admitted that was the case. In addition to such cases of complete developmental inhibition, several cases were found where mM amounts of IAA caused ethylene production, with the ethylene in turn inducing flowering (*e.g.*, the bromeliads). These auxin effects are presumably pharmacological not physiological.

The early research on the effects of added auxin was reviewed by LANG in 1961. Research since then has been summarized in the volumes by BERNIER.

et al. (1981). Inhibition of flowering has been the most frequently observed effect of auxin. In most cases the photoinduced leaf seems to be the site of auxin action rather than the later transforming shoot apex. This conclusion is based on both the greater effectiveness of IAA that has been added to the leaf compared to the shoot-tip (*e.g.*, OGAWA 1962) and on timing studies in which IAA inhibits most when given to a leaf just before or during the inductive photoperiod (*e.g.*, SALISBURY 1955, OGAWA 1962, EVANS 1964) — *i.e.*, before the floral stimulating effect has presumably reached the apex. For SDP *Chenopodium*, however, IAA added at 0.1 mM inhibited flowering when added to the shoot tip, so long as it was applied close to the time of the inductive photoperiods (SEIDLOVÁ and KHATOON 1976) and concomitant anatomical studies of the shoot apex convinced them that the added IAA was inhibiting flowering by increasing apical dominance — release from which marked the start of floral differentiation. A nice series of experiments by KREKULE and PŘIVRATSKÝ (1976) using ^{14}C-IAA confirmed the view that even IAA added to the cotyledon of *Chenopodium* was actually inhibiting flowering by moving to the shoot tip.

The high concentrations of IAA added in these studies of floral inhibition — typically 0.1 to 1.0 mM — are worrisome even though the volume added is sometimes small. As SALISBURY pointed out in his book (1963), such quantities cause wrinkling of laminas and twisting of petioles in treated leaves of *Xanthium* — effects apparently pharmacological.

To allay this anxiety about the concentrations of IAA or other auxins added, convincing evidence about the extent to which the added auxins exactly substitute for endogenous auxin would be valuable. At present, such evidence seems to be nonexistent. The data that most nearly approach this desideratum came from several papers on the Princeton clone of *Coleus blumei*, a day-neutral plant. Excising axillary buds and branches caused compensatory growth of the main shoot and also speeded flowering; substituting 1 % IAA in lanolin for the axillaries slowed flowering once again, but did not affect the compensatory growth (JACOBS *et al.* 1959; *cf.* also ALONI 1976). Other research on this clone provided evidence from auxin bioassays, tracheary regeneration and abscission studies that 1 % IAA in lanolin provides auxin into *Coleus* shoots at a level that matches that provided by the endogenous sources (summarized in JACOBS 1979). However, the inhibition of flowering by IAA was relatively slight (only 7 days later than the 69 days to flowering observed in the control group) (JACOBS *et al.* 1959).

Studies of endogenous auxin in plants undergoing floral induction have been almost all based on bioassay measurements. Several investigators provided data on plants that were already fully induced and hence were not relevant to our interest in the early stages. The first people to investigate endogenous auxin levels reported auxin acitivity in their extracts as though it were due to IAA. As separation methods improved, paper- and thin-layer-chromatotography was used, with ^{Rf}IAA^{Rf}* being followed (*e.g.*, BRONCHART 1957, GILSON 1957). There was, apparently, an unspoken assumption that IAA, and only IAA, was the endogenous auxin.

For other types of plant hormone, for a long time there has been evidence for multiple forms whose relative proportions change as floral development

* This notation is a convenient and condensed way to refer to IAA-like activity occurring at the Rf typical of IAA on thin-layer or paper-chromatograms (JACOBS 1979).

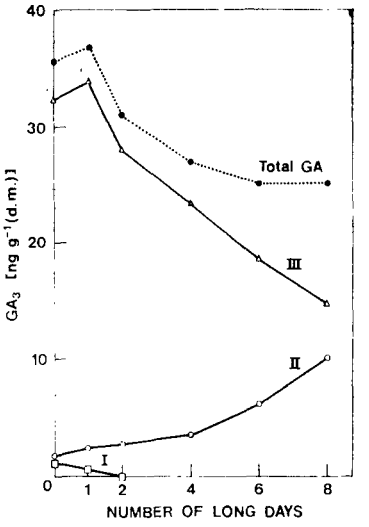

Fig. 1. The effect of increasing numbers of LDs on the gibberellin activity at 3 zones of chromatograms of LDP *Spinacia* extracts (JACOBS 1979, after ZEEVAART 1971).

proceeds. ZEEVAART's work (1971) on three ^{Rf}gibberellins^{Rf} in photoinduced LDP spinach is a striking example (Fig. 1). Even more striking changes in multiple zones of hormone activity had been found in extracts of LDP *Rudbeckia* after successive weeks of photoinduction (HARADA and NITSCH 1959) (Fig. 2), although they used a bioassay that responded to both gibberellins and auxins. Eluates of the 'E' zone (which provided maximal bioassay activity one week before bolting occurred under long days) also caused bolting when added to plants in a non-inductive photoperiod. Several ^{Rf}cytokinins^{Rf}

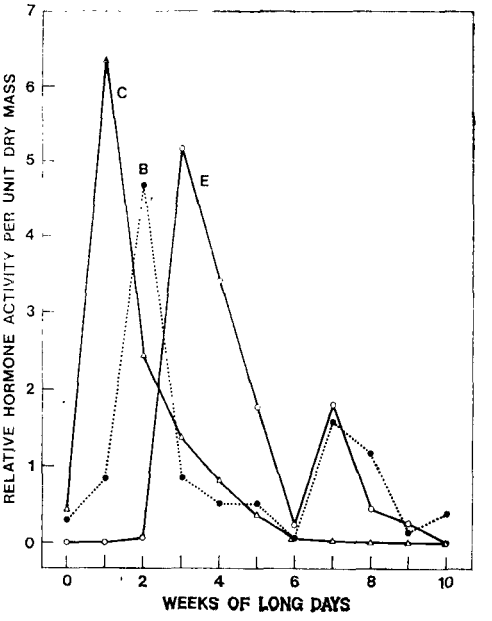

Fig. 2. The effect of increasing weeks of LDs on the auxin-gibberellin acitivity at 3 zones of chromatographed extracts of LDP *Rudbeckia* (JACOBS 1979, after HARADA and NITSCH 1959).

have been found in each of many plant extracts. Direct-probe mass spectrometry has confirmed the multiplicity of endogenous cytokinins (ENTSCH *et al.* 1980). In some cases these endogenous cytokinins have been causally related to developmental changes. For example, 3 such active zones were reported from radish roots (RADIN and LOOMIS 1971) with only 2 of the

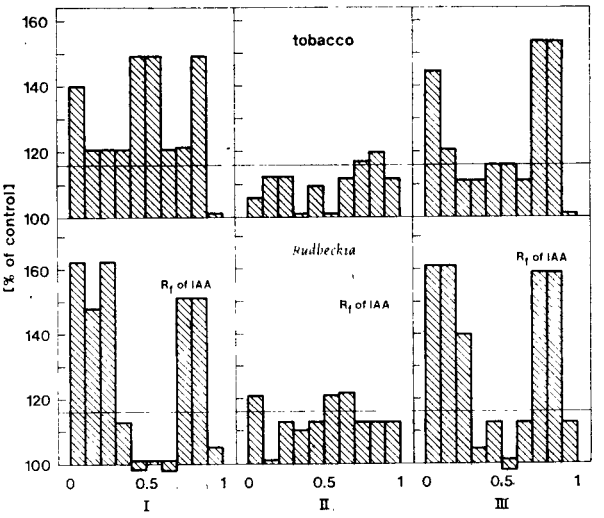

Fig. 3. Auxin activity from various zones of chromatographed extracts of SDP Maryland Mammoth tobacco (top) and LDP *Rudbeckia* (bottom). Photoperiodic conditions were: I — 16-h day; II — 8-h day; III — 6-h day and 2-h night break (from CHAĬLAKHYAN and LOZHNIKOVA 1966).

cytokinins increasing at the same time as the cambial activity, which they could induce in culture. The third ^{Rf}cytokinin^{Rf} did not increase until later and could not induce cambial activity.

I would suggest that the time is overdue for us to consider seriously the possibilities: 1) that auxin is like other plant hormones in having more than one chemical form in a given plant; and 2) that a likely time for auxins to appear or disappear is during the change from vegetative to reproductive development. Is there any evidence to support this view? If we look through published figures of chromatograms assayed for auxin-activity, we can find evidence of new ^{Rf}auxins^{Rf}. For instance, Figure 3 shows such data for extracts of SDP Maryland Mammoth tobacco under LD and SD conditions: under the conditions that prevent flower-induction, a new ^{Rf}auxin^{Rf} is present. Extracts of the LDP *Rudbeckia* do not show this ^{Rf}auxin^{Rf} under either LD or SD. By ignoring zones of auxin activity other than those at the Rf of IAA, we may have been needlessly limiting our understanding of floral development. The new ^{Rf}auxin^{Rf} in Figure 3, for instance, may function as an endogenous floral inhibitor (evidences for which were summarized recently by JACOBS 1980, and LANG 1980). [There is no reason to think that non-IAA auxins will be limited to stages of floral development: WIGHTMAN and his co-workers, for example, have presented increasingly convincing evidence for phenylacetic acid as an endogenous auxin along with IAA, culminating

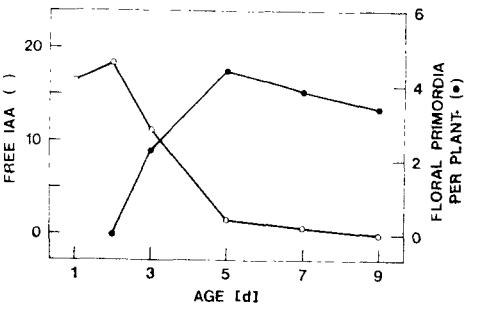

Fig. 4. Response of SDP *Pharbitis* to SD at various days after sowing as measured by the number of floral primordia per plant and the amount of auxin activity recovered at the Rf of IAA from ether extracts of cotyledons of various ages (numbers represent average *Avena* curve). Data from OGAWA (1962).

in GC-MS identification of these compounds from tobacco shoots (WIGHTMAN and LIGHTY 1982).]

Despite the need for actual chemical identification of the various endogenous auxins, what can we learn from the earlier studies of ^{Rf}IAA^{Rf} that were limited to bioassays? GILSON (1957) reported that the level of ^{Rf}IAA^{Rf} in LDP *Hyoscyamus* did not differ under SDs and LDs until after induction. OGAWA (1962) measured endogenous levels of ^{Rf}IAA^{Rf} in extracts of SDP *Pharbitis* cotyledons, in relation to changes with age in flowering response to a SD. Data selected from two of his graphs are re-assembled in Figure 4, which reveals a high level of "free auxin" (ether-extractable) in cotyledons of seedlings one or two days old, decreasing to vanishingly small amounts by day 5. Response to SD, as measured by number of floral primordia, shows an intriguing inverse parallel variation, with no response at day 2 and with the response increasing to the maximal number of floral buds at day 5. Added IAA inhibited flowering in *Pharbitis* (as one example from Ogawa's extensive data, 5 mg IAA l^{-1} reduced the number of floral primordia per plant from 6.4 in the controls to 1.5 in the IAA-treated). IAA was much more effective when added to the cotyledon than when added to the shoot-tip, fitting the view that its primary site of action was in the cotyledon. These results, as far as they go, seem to fit the hypothesis that endogenous IAA is inhibiting the flowering response in *Pharbitis* cotyledons. Rather surprisingly, however, OGAWA concluded that there was "no evidence which suggests a decisive role of endogenous auxin in floral induction." Apparently this conclusion was partly based on his finding no significant change in levels of endogenous ^{Rf}IAA^{Rf} of 8 or 9 day-old *Pharbitis* plants that had recently been given 3—5 SDs as contrasted to those kept in continuous light.

But the role as an endogenous inhibitor of auxin (or, indeed, of whatever substances were responsible for the graft-transmittable floral inhibitions reported by LANG *et al.* [1977] in LDPs or by JACOBS [1980] in SDPs) does not require that the level should rise and fall with the non-inductive and inductive photoperiod, respectively. The inhibitory substances may be providing a general background of inhibition which the stimulating effects of the inductive photoperiod can overcome. From the evidence available it seems that, if IAA is normally involved in inhibiting flowering, it is doing so as such a general "background inhibitor."

One other line of IAA investigation has been applied to the flowering problem more recently: the possibility that the polarity or transport-capacity of tissues for IAA might change with photoinduction. The amount of IAA that stems or petioles were able to transport (as separate from the amount

normally available to them) has been shown to be a limiting factor for several developmental processes in vegetative *Coleus* (*cf.* JACOBS 1979), and it was hypothesized that photoinduction might significantly change polarity or transport capacity — a change that might not be reflected in amounts of IAA found in extracts. However, the hypothesis was not confirmed: there was no detectable change in the polarity or amount of IAA movement through SDP *Xanthium* petiole-sections at various stages of induction (JACOBS 1978).

One final caution I would like to add: direct measurements of auxin levels are much to be preferred to indirect ones. As an example, several scientists have reported on the gradient of flower-forming activity in cultures from different locations on tobacco stems (*e.g.*, AGHION-PRAT 1965). The effects of added IAA could lead one to expect that a gradient of endogenous IAA was involved (*e.g.*, WARDELL and SKOOG 1969). But a detailed study of endogenous IAA levels, using capillary gas chromatography and selected ion monitoring with a mass spectrometer, and with deuterium-labeled IAA as internal standard, revealed no gradient of endogenous IAA (NOMA *et al.* 1984).

So, in summary I would interpret available evidence in favor of endogenous IAA normally acting as a "background inhibitor" of flowering, but I feel we need to use available physico-chemical methods much more generally to investigate not only IAA levels inside the plant, but also the levels of other auxins present at various times during development.

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BOOK REVIEW

BÖHMER, B.: RATGEBER FÜR PFLANZENSCHUTZ UND UNKRAUTBEKÄMPFUNG IM ZIERPFLANZENBAU. — Verlag Paul Parey, Berlin — Hamburg 1985. 187 pp. Kartoniert DM 34.00.

This handbook contains recommendations for controlling the disease, pest and weed in ornamental plants. Importance of the efficient control increases with the increasing intensity of growth of ornamental plants and with its concentration. Early diagnosis of the causal agent of the disorder is stressed. Aspects of the integrated plant protection are pointed out: chemical control should be considered in connection with other plant health supporting measures. Therefore much attention is paid to the prevention from the spread of pests and diseases. Diagnosis of the causal agent of the disorder is based on symptoms grouped into five basic categories. They correspond with the description of single pests and diseases in the following chapter. This arrangement considerably facilitates the determination of the disease. Ornamental plants are listed in alphabetic order according to their Latin names followed by German equivalents. Optimum pH values and critical salt concentrations (in grams per litre) are given for each ornamental plant, then diseases and pests are enumerated. Recommended control measures are attached or reference to the page where they are described is given. Data on intolerance of pesticides are also included. Control of the most common pests, diseases and weeds as well as directions for application of the most common pesticides and herbicides are described in the following two chapters. The last two chapters deal with the necessary precautions in pesticide application.

The publication is surely one of the most modern handbooks on pest, disease and weed control in ornamental plants not only for the ample amount of condensed information but also for the practical approach used.

P. BARTOŠ (*Praha*)