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Plant hormones and the control of physiological processes

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Contents

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Summary

This review examines contemporary views of the role of plant hormones in the control of physiological processes. Past and present difficulties with nomenclature encapsulate the problems inherent in using the 'classic' hormone concept in plants, with their distinctive multicellular organization. Chemical control may be a more relevant notion. However, control may also reside in the responding tissue via changes in sensitivity, or as combined control, where response is dictated by both sensitivity and concentration. Criteria for demonstrating these modes of action are reviewed, as well as frameworks for deciding whether hormone transport is involved. Problems of measuring relevant hormone concentrations are discussed. Methods for measuring and comparing tissue sensitivity to hormones are outlined and relative control is introduced as a means of assessing the importance of hormonal control against a background of other influences. While animals and plants appear to have coinherited homologueous intracellular signalling systems, at the whole organism level modes of hormone action may diverge. It is postulated that the synthesis-transport-action mechanism of action may be just one of several possible ways that phytohormones could control physiological processes. Twelve separate roles are discussed, and it is suggested that some of these could operate simultaneously to the plant's advantage.

Abbreviations

C_i, intracellular CO_2 concentration; D-R, dose–response, F, factor; H, hormone molecule; $[H]_n$, value of [H] at $R = R_{MIN} + (n/100)R_{AMP}$ (e.g. $[H]_{50}$, value of [H] at $R = R_{MIN} + 0.5R_{AMP}$); R, initial rate of response following exposure to hormone concentration [H]; R_{AMP}, difference between R_{MAX} and R_{MIN}; R_{MAX}, the maximum initial rate of response (obtained theoretically at infinite [H]); R_{MIN}, minimum initial rate of response (obtained in the absence of added H); Rec, hormone receptor.

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I. Introduction

The plant hormone concept dominated and shaped experimental plant physiology for much of the 20th century. Indeed, at certain times, the volume of publications on phytohormones dwarfed that on either the biochemical or biophysical aspects of the subject. Molecular biology now dominates both funding and journal space and the plant hormones *per se* do not receive as much attention as they used to. However, important issues about hormone action were not resolved satisfactorily when the topic held sway. As a result, there is the air of an 'unfinished project' about the phytohormone research field. This review starts from the position that the reasons why plants have hormones is not fully understood and doubts remain as to how they exert control over plant actions at the whole organism level.

Modern molecular and genetic analyses provide superb opportunities for understanding many details of the mechanism of hormone action, and relevant techniques are increasingly being applied in this field (Leung & Giraudat, 1998; McCourt 1999; Bleecker & Kende, 2000; McAinsh *et al.*, 2000). The publication of a whole plant genome (The *Arabidopsis* Genome Initiative, 2000) heralds an explosion of knowledge regarding metabolism, receptors and mechanisms, based on comparisons with better-understood organisms, reconstruction of proteins and, of course, on manipulation of the genome and its products. It is important that such work is based on a realistic view of the potential role(s) of plant hormones, and it is vital to recognize at the same time that knowledge of molecular events alone cannot provide complete answers to all questions, especially those framed at the whole organism or community scale.

Because of the past volume of research on phytohormones, there have been many reviews covering their chemistry and action, and several treatments have, necessarily, been encyclopaedic (e.g. Steward, 1960; MacMillan, 1980; Scott, 1984; Letham *et al.*, 1978; Takahashi, 1986). This review will not cover this body of work in any detail. Instead, the aim is take a step back from detailed studies of mechanisms of plant hormone action to focus on 'whole system' issues and in particular on the conceptual models underlying plant hormone research. The last major reviews of hormonal function in plants (MacMillan, 1980; Scott, 1984; Hoad *et al.*, 1987), were completed before or during a phase of some controversy (Trewavas, 1981; 1983a, 1991; Cleland, 1983; MacMillan, 1987) and many contentious issues raised then are still unresolved. Furthermore, this debate stimulated several new approaches (Nissen, 1985; Firn, 1986; Trewavas, 1991; Weyers *et al.*, 1987; Weyers *et al.*, 1995; Paterson *et al.*, 2001) which have yet to be fully discussed and integrated into the field.

The primary objectives of this review are thus to:

- analyse the historical development of the plant hormone concept, and in so doing, clarify the key concepts and terminology involved;
- explore the conceptual frameworks that have been proposed to support research in this area;
- discuss criteria by which candidates for hormone status can be assessed, both for new compounds that may be identified, and for old ones in 'established' and newly considered physiological processes;
- review specific technical and physiological issues relating to the collection of evidence within these guidelines;
- discuss issues relating to hormone sensitivity and relative control potential and explain their key importance for an understanding of hormone action;
- review the full range of roles for plant hormones in plant physiology and why these might have evolved;
- more generally, help pinpoint relevant hypotheses, improve the design of experiments and facilitate the interpretation of results within a holistic context.

Although many readers may be familiar with the history of the field, this will first be presented in condensed form to prepare the context for the ensuing discussion.

II. The history of the hormone concept in plant systems

The hormone concept arose from the work of the mammalian physiologists Bayliss and Starling on the influence of the small intestine lining on pancreatic secretions following the ingestion of food. It was Starling (1905) who coined the term hormone from the Greek '*horman*' (to stimulate) and the compound involved is now known to be the polypeptide secretin. The hormone concept in its original ('classic') sense thus involves three fundamental elements:

1 synthesis of a chemical influence in one organ;
2 transport (via the bloodstream in the mammalian case);

3 action of the chemical action in a second organ to control a specific physiological event.

Although 19th century plant physiologists such as Sachs had written of 'organ forming substances' (see Meidner, 1985), it was Fitting (1909, 1910) who was the first to use the term 'hormone' in a botanical context, adopting it to describe post-flowering phenomena in orchids. Paál (1919) embraced the hormone concept in his work on phototropism, but he did so without actually using the word (he wrote of a 'correlation carrier'). However, it was the discovery of the role of the grass coleoptile tip in phototropic behaviour that provided the main impetus for the adoption of the hormone concept in plant biology. Darwin & Darwin (1880) discovered that a decapitated coleoptile will not exhibit a phototropic response to unidirectional irradiation, and a series of further experiments indicated that its influence can be passed on across a permeable barrier (Boysen-Jensen, 1913, Paál, 1919). These results, combined with those from many subsequent studies, implied that a chemical influence from coleoptile tip might influence growth in lower parts, thereby controlling the response to directionally biased irradiance.

During the 20th century, a series of naturally occurring compounds with physiological activity were discovered, and, both in texts and in the minds of researchers, the most important were classed as plant hormones (or some synonym, see section III) and grouped according to chemical structure and/or physiological effect. Listed in order of the date of their first unequivocal identification in a plant tissue, the most notable include: ethene/ethylene (Gane, 1934); the auxins (IAA – Haagan-Smit *et al.*, 1942); the gibberellins (GA_1 – MacMillan & Suter, 1958); the cytokinins (Zeatin – Letham, 1963); abscisic acid (Cornforth *et al.*, 1965); the brassinosteroids (brassinolide – Grove *et al.*, 1979); the jasmonates (jasmonic acid – Ueda & Kato, 1980); and the polypeptides (tomato systemin – Pearce *et al.*, 1991). Other groups with physiological activity such as the polyamines (Smith, 1985) and the oligosaccharins (Aldington & Fry, 1993) have also been identified. Essentially, a wide range of plant secondary products have effects on plants (Bearder, 1980, Takahashi, 1986).

Even as these physiologically active phytochemicals were being identified, the simple notion of plant hormones acting like those in animals began to break down. Although these compounds were capable of influencing development, they did not always appear to have a clear locus of synthesis. Many were identified in plant translocation systems (Kaldewey, 1984), but there was only patchy evidence that they might be transported as an integral part of their mode of action. Many (if not most) physiological systems appeared to respond to more than one chemical signal and each compound could affect many systems (Matthysse & Scott, 1984). There were systems that appeared to respond according to the balance of compounds in the tissue (Wareing, 1977). There were also systems where physiological activity appeared to be dictated not by changes in concentration at the site of action, but by spatial differences or temporal changes in the tissue's sensitivity to the compound (Trewavas, 1981).

In retrospect, the simple 'synthesis-transport-action' animal hormone model never appeared to fit the plant case well, but there was little acknowledgement that a different kind of thinking was required. Plant scientists appeared to be content with the notion that 'special' chemicals were involved in the control of physiological phenomena, even though no clear explanatory model took the place of the classic hormone one. Trewavas was the first to point this out in robust terms (Trewavas, 1981). The many issues introduced by Trewavas in that and subsequent reviews will be discussed later at various points in this review. However, one of the most fundamental questions he asked was whether the transfer of concepts between animals and plants was valid. He suggested that problems with the adoption of the classic hormone concept were due to the fundamental differences between the two kingdoms. This remains an important starting point for any debate on the role of plant hormones in the control of physiological processes.

In comparing plants with animals, it must be acknowledged that these groups separated in evolutionary terms at the single cell stage. Thus, some of the essential differences between the kingdoms (e.g. in organelle complements, cellular anatomy or basis of nutrition) might lead one to assume independence of systems for dealing with problems of multicellularity (Trewavas, 1981; Meyerowitz, 1999). It should also be noted of course that higher animals have a well-developed nervous system that complements hormonal control. Plants may have evolved to replace some of the roles of nerve connections in animals by chemical messages.

The evolution of animal hormone systems is correlated with important phylogenetic events during evolution. Notable examples include the development of functional moulting hormones around the time of the split of Protostomia into the Ecdysozoa and Lophrotochozoa (Aguinaldo *et al.*, 1997)[1] and the evolution of male and female sex hormones together with gene duplication episodes during vertebrate evolution (Hawkins *et al.*, 2000). So, on the animal side, at least, there is strong evidence for the evolution of new hormone systems, after the plant-animal divide. Why not on the plant side also? Important features of plants that might have influenced the independent evolution of phytohormone systems include:

- Possession of a cell wall and plasmodesmata. The cell wall might initially appear to be a barrier to cell-to-cell communication via chemicals, but most cells in the plant body are joined by plasmodesmatal connections (Ehlers & Kollmann, 2001), with the notable exception of mature stomata (Sack, 1981). Plasmodesmata may play an important role in dissemination

[1]Intriguingly, a range of phytoecdysteroids have also evolved in plants as a chemical defence against insects but probably independently (Jones & Firn, 1978; Grundvald, 1980).

of chemical signals over short distances, including macromolecules (Oparka & Santa Cruz, 2000). Animal cells lack such symplasmic connections, although they do have mechanisms for communicating between cells (e.g. nerve gap junctions) that are well understood. If electrical continuity exists between all plasmodesmatally connected plant cells (Spanswick, 1972), this would imply that a hypothetical electrical (i.e. nerve-like) form of control in plants would be a less effective means of delivering a signal than it is in animals. This might favour the evolution of specialized chemical control systems, although a more likely benefit can be seen from avoiding the need for preprogrammed (i.e. nonplastic) development. Nevertheless, while outwith the scope of this review, electrical and other physical forms of signal transmission cannot be ruled out entirely (Davies, 1987; Malone, 1996);

- Nature of supracellular transport systems. Plants and animals have different mechanisms for generating the pressure differences that drive their mass flow circulation and transport systems. In itself, this is likely to be of lesser importance than the fact that the plant systems are more open-ended and have a tendency for unidirectionality (at least at the conduit level), rather than operating in a continuous circulation circuit. This enhances the likelihood of delivery of a chemical message to a particular organ (e.g. to leaves from roots in the xylem; to carbohydrate sinks from leaves in the phloem). On the other hand, directionality and speed of plant circulatory systems are influenced by physical conditions (Nobel, 1991) and this would reduce the reliability of the corresponding communication pathway (see section VII);
- Plastic rather than canalized development, controlled by meristems. Plant responses to external challenges and opportunities are via growth and development rather than by the motile behavioural mechanisms which have evolved in animals (Jennings & Trewavas, 1986). By contrast with nervous systems, hormonal control systems do not need to be 'hard wired' developmentally in terms of a direct system of communication between specific cell groups. Thus, while whole organism motility and behavioural diversity is a benefit to animals in general, few 'higher' animals are capable of flexible responses involving development. In plants, these are common mechanisms. Examples include regeneration (e.g. as following herbivory), redirection of growth (e.g. as in apical dominance) and developmental response to environment (e.g. etiolation). For success of the individual, these different response systems need to be coordinated and the activities of different meristems integrated, suggesting a potential role for phytohormones (Trewavas, 1986a);
- Timescale. The generally longer lifetimes and slower pace of growth and development in plants means that they do not require a very rapid (e.g. nervous) form of control. Consequently, plants have evolved nerve-like message-carrying systems in only a restricted number of plant species and only when speed of reaction has special advantages, for example in the triggering of the *Dionaea muscipulata* trap and communication among *Mimosa pudica* leaves and leaflets (Salisbury & Ross, 1992). Long-lived chemical signals might be better placed to integrate activity over longer timescales. Bradford & Trewavas (1994) introduced the concept of 'biotime' in considering the action of plant hormones. They noted the flexibility of 'physiological time' in relation to developmental events and suggested that, alongside other inputs, variations in hormone sensitivity and concentration could play an important role in regulating this (see section XI).

Looking at plants and animals as systems, these innate differences can be contrasted with similarities in some physiological requirements. Both groups utilize homeostasis for efficient operation, although they do not necessarily regulate the same parameters. The feedback loops associated with this are frequently hormonal in animals, and they may also be in plants. A postulated example is the feedback loop involving ABA that is thought to maintain leaf turgor (Raschke, 1979, see section XI). In both sets of organisms, there is also the need to coordinate the activities of cells at the tissue and organ level to provide efficient responses to stimuli. In complex systems, hormones might be well suited to this form of control, where the locations being controlled may be scattered. They also allow a certain degree of spatial flexibility in response: chemical control offers the possibility for responses to be locally tuned via tissue sensitivity (as in the 'target cell' concept, see sections IX and X).

Hormone receptor and transduction systems in multicellular organisms could have evolved by modification of the chemotactic sensing systems involved in feeding and sex ('external sensors', Meyerowitz, 1999). Trewavas (1981) speculated that the coding for these molecular systems might already have been present in single cells, as seen in the integration of cellular activity within microbes by cAMP. Intriguingly, the concept of 'quorum sensing' in microbes suggests that these minute cells may be able to signal to each other using chemical messages to coordinate their activity (Swift *et al.*, 1996). Apparently, at the point of their phylogenetic split, animals and plants coinherited a similar genetic library or 'construction kit' of membrane-associated proteins capable of acting as receptors and transducers of signals, and similar systems capable of acting as second messengers or cascade-amplification mechanisms. Plants inherited additional signalling elements of cyanobacterial origin (The *Arabidopsis* Genome Initiative, 2000). At the cellular level, the homologous elements of plant and animal signalling systems seem to have been well conserved – certainly, detailed comparison of plant, animal and fungal homologues of the kinase elements of signal transduction pathways (Hardie, 1999) gives this impression. At the whole organism level, this genetic information has probably provided the material to give rise independently to analogous responding systems (Meyerowitz, 1999).

It is apparent to any observer of research in animal and plant hormone systems that scientific knowledge of animals is more advanced. There is value in exploring briefly why this

should be so, because it reveals important aspects of the politics and economics underlying investigation in the two fields. In the first place, the medical dimension of animal hormone work has provided both motivation and funds – certainly in the mammalian examples. The effect of this on the sheer volume of work by itself would alone have stimulated progress, but, in the case of the mammals at least, the physiological systems themselves provide additional advantages. Blood as a translocation medium, for example, is readily sampled in reasonable volume and the easily separated serum component provides a relatively pure starting point for physicochemical analysis (Dacie & Lewis, 1995).

By contrast, it is notoriously hard to sample phloem and xylem sap due to their inherent physical nature and physiological protection mechanisms (Eschrich, 1975; Ziegler, 1975; Pate, 1976), while the transport systems for polar transport remain somewhat hypothetical (Kaldewey, 1984). Further, physical manipulation of animal systems is easier: the secretory organs and glands of animals develop as discrete entities whereas plants tend to exhibit a nondiscrete form of differentiation. Animal secretory organs often have ducts, which have facilitated experimental manipulation. Also, when analysing samples for putative hormone content, the chemical milieu in plants tends to be more complex owing to the presence of the numerous and diverse products of secondary metabolism (Yokata *et al*., 1980; Reeve & Crozier, 1980).

Against this, plant systems can be seen as advantageous in terms of the possibilities for manipulation of the genes controlling plant hormone physiology. While legal, bureaucratic and safety issues cannot be ignored in either field, these official requirements tend to be less onerous for plants than they are for 'higher' animals. Systems for transforming the genome and for looking at the effects in the whole organism are certainly easier to adopt in plants. The knowledge base in this area is expanding rapidly and model systems such as *Arabidopsis*, with its small genome and relative ease of transformation and propagation, offer much scope for future research.

Looking back, the early researchers on plant hormones, fired by their initial successes, were optimistic to the point of naivety. Thus, at least with respect to hormonal control of tropisms, Cholodny (1927) was confident enough to state: '... there emerges from this chaos of facts the splendid form of a theory which promises to unite and to coordinate, in the very near future, the enormous mass of varied experimental data into a single principle.' Commenting on this in their book of 1937, Went and Thimann felt able to say 'This prophetic statement has to a large extent come true'. Today's workers in that field would certainly not agree unanimously with this viewpoint and the matter remains unresolved (Firn & Digby, 1980; Digby & Firn, 1981; Firn & Myers, 1987; Wheeler & Salisbury, 1981; Evans, 1992; Iino, 1992; Firn, 1992; Salisbury, 1992; Trewavas, 1992; Edlemann, 2001).

Tony Trewavas' seminal review 'How do plant growth substances work?' (Trewavas, 1981) focused discussion on what he regarded as the essential failure of the hormone concept when applied to plant systems. He questioned a number of assumptions generally made by plant hormone researchers, especially the premise that changes in concentration alone controlled plant responses. Trewavas followed up this analysis with several other papers (Trewavas, 1981, 1982, 1983a, 1983b, 1986a, 1986b, 1987, 1991; Trewavas & Jones, 1981; Hanson & Trewavas, 1982; Bradford & Trewavas, 1994) and the debate continued both at meetings and in publications (Radin & Ackerson, 1982; Cleland, 1983; Weyers, 1994; Trewavas, 1983a, 1987; MacMillan, 1987).

The main points made by Trewavas (1981) were as follows:

- despite many years of work, no coherent picture had emerged concerning the role of plant growth substances;
- during this time, little account had been taken of the differences between plant and animal development and organization;
- researchers had been sidetracked by the analogy with animal hormones in terms of the notions of 'action at a distance from synthesis' and 'control via changes in concentration', whereas for plants, there was evidence that sensitivity to hormones might more important;
- when critically examined, much of the detailed evidence in plants does not support this model, or at best does not explain the true complexity of plant responses;
- there is a mismatch between observed dose–response curves for applied compounds (which generally cover at least two orders of magnitude and often more) and the changes in endogenous hormone concentrations observed within plant tissues which were much smaller at two-10-fold. This, Trewavas felt, meant that hormones would be unlikely to control events via changes in their concentration.

In a later commentary, Trewavas (1983a) pointed out additional difficulties for plants in accurately controlling hormone concentration at their presumed site of action.

Instead of a classic hormonal role, with specific sites of synthesis and action, Trewavas has suggested at least four other modes of action:

1 as integrating and smoothing agents in development, counteracting disorganized gradients or timing of resources (Trewavas, 1981; 1983b);

2 as integrators of wound responses (Hanson & Trewavas, 1982);

3 as controllers of resource allocation under poor growth conditions (Trewavas, 1986a);

4 as regulators of physiological time, or 'biotime' (Bradford & Trewavas, 1994).

While there was a degree of adverse comment following Trewavas' intervention, there can be no doubt that attitudes changed thereafter (Salisbury & Ross, 1992), as can be seen both in the tenor of reviews and texts appearing over the relevant period, and their direct and relatively lengthy reference to the issues (e.g. Roberts & Hooley, 1988). Others took the opportunity to explore other control possibilities, such as action potentials (Davies, 1987).

Cleland (1983) and MacMillan (1987), responding to Trewavas' points, contended that there was evidence for action at a distance in specific systems, such as xylem differentiation in the stem (auxins), α-amylase production in barley seed germination (gibberellins) and root gravitropism (abscisic acid). Cleland also cited cases where there did appear to be a quantifiably reasonable concentration-related effect. In later papers, Trewavas has contested some of these interpretations – from a neutral standpoint, the evidence in the literature can apparently be selected to support either side's view. Hence, in presenting this issue to students, Mohr & Schopfer (1994), were content simply to subdivide the actions of hormones into cases where transport occurred and those that where it did not.

Trewavas has been criticised for holding the extreme view that sensitivity is the primary controlling factor in plant responses (Cleland, 1983; MacMillan, 1987; Roberts & Hooley, 1988). Zucconi (1988) commented that this was effectively a parallel regression to the concept that control may be accounted for by single determinants (although Trewavas has also championed quantitative consideration of other influences – see section X). Cleland (1983) accepted the potential role of sensitivity in hormone responses and pointed out that this had been implicitly acknowledged by early hormone researchers, and from time to time explicitly brought to the fore, both in the primary literature and in reviews (Went & Thimann, 1937; Thimann, 1948; Wright, 1966; Audus, 1969). Significantly, Cleland (1983) put forward the notion that control might be exerted either by concentration changes or by alterations in tissue sensitivity, and possibly by a combination of the two. Thus, the truth could lie somewhere in between the extreme views (Cleland, 1983; Weyers *et al.*, 1987, 1995) although the adaptive benefits of this situation for plants is not yet fully understood.

Notwithstanding this continuing debate, a simplified treatment of hormone physiology regrettably continues in the plant physiology presented in school curricula and in many general university texts. At this level, complicated issues are often sterilized and reduced in complexity to the point of portraying systems and results in a cartoon-like manner, and there is a risk that ideas absorbed at an early stage may influence later attitudes. There is a need for recognition of the complexity involved in phytohormone responses (some of which is illustrated in Fig. 1) and an understanding that all experiments must be carefully designed and results carefully interpreted within this context. Following a brief diversion into nomenclature that incorporates discussion of certain key issues in hormone action, the development and current status of frameworks for analysing hormone involvement in physiological processes will be reviewed.

III. Issues of nomenclature

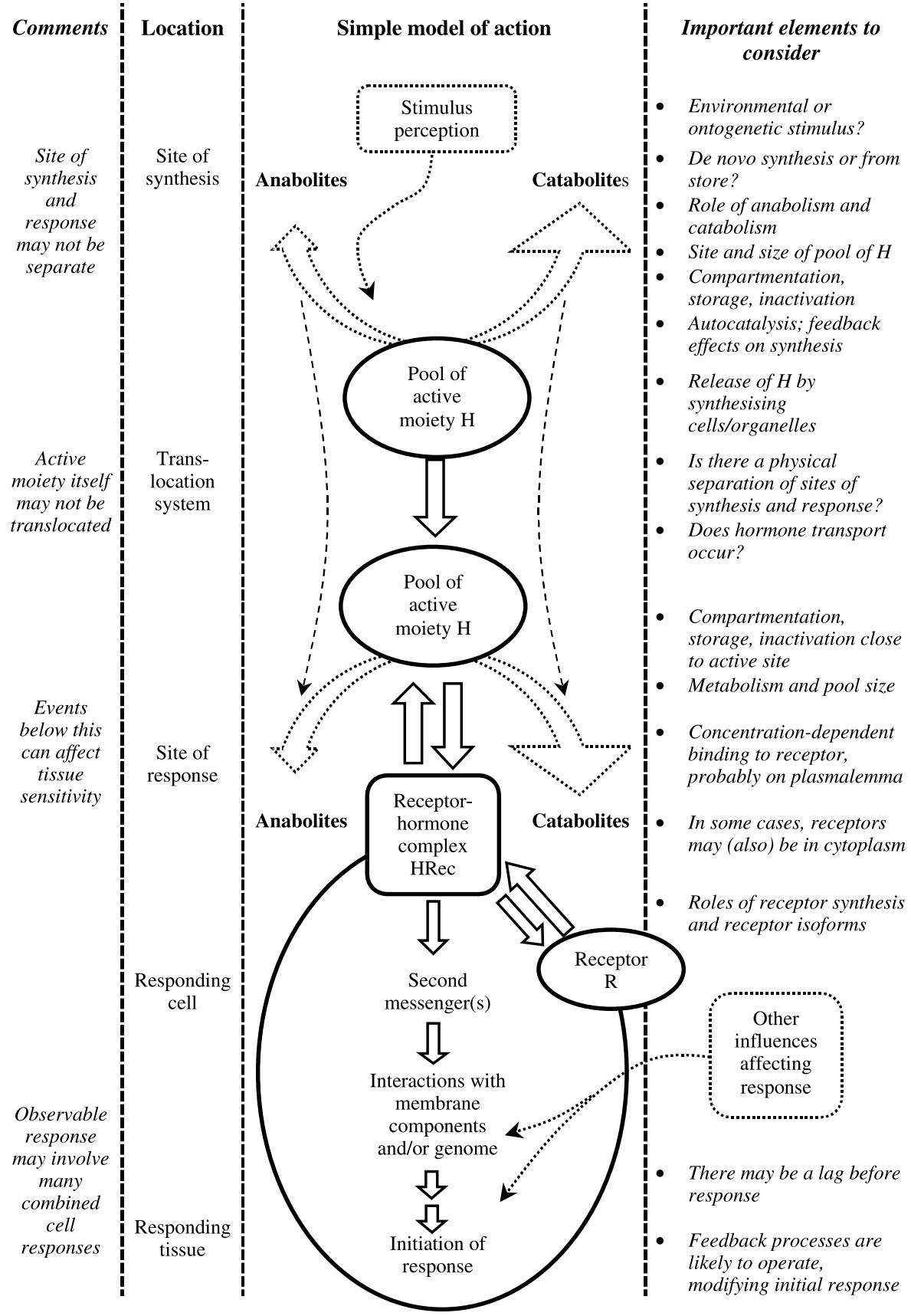
Historically, there has been a fair amount of disagreement regarding appropriate nomenclature within the plant hormone field. To some extent, this reflects or is symptomatic of differences of opinion about the underlying concepts. Trewavas (1986a) claimed that 'mistaken terminology' was a reason for adopting what he felt was an unsatisfactory model for research, so it is important to clarify the issues involved. The following is a brief synopsis of this debate as it occurred in the last century.

As noted above, the word 'phytohormone' was introduced at an early stage to distinguish plant hormones from those of animals, but several other names have since been preferred by specific authors. The synonyms proposed include: 'plant regulators', 'plant growth regulators', 'plant growth hormones', 'plant growth substances' (discussed by Tukey *et al.*, 1954), and the more esoteric 'ergons' (Larsen, 1955), 'bios' (Löve & Löve, 1945), 'correlation carriers' (Paál, 1919), 'semiochemicals' (Birch, 1974) and 'enablers' (Barlow, 1987). An attempt was made to propose a uniform defined nomenclature (Tukey *et al.*, 1954), but agreement was by no means universal (Larsen, 1955; Audus, 1972).

The definition of 'hormone' in Webster's Dictionary (Gove, 1961) is: 'a specific organic product of living cells that, transported by body fluids or sap, produces a specific effect on the activity of cells remote from its point of origin'. Pincus & Thimann (1948) considered that a 'phytohormone' was 'an organic substance produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its site of action, and active in minute amounts'. According to these definitions, man-made compounds not known to occur in plants cannot be termed hormones, so the terms 'plant regulators' and 'plant growth regulators' were proposed by Tukey *et al.* (1954) to include natural and artificial compounds. They defined 'regulators' as 'organic compounds, other than nutrients, which in small amounts promote, inhibit or otherwise modify any physiological process in plants'.

This term was objected to by Larsen (1955), who claimed that the established definition of the verb 'to regulate' was to 'adjust so as to work accurately and regularly'. He maintained that many of the man-made compounds did not fulfil this definition in their effects, and proposed the terms 'growth substance' and 'growth hormone' to cover artificial and endogenous compounds, respectively. Based on alternative definitions, Cram (1976) drew a distinction between the terms regulate and control, stating that 'regulation' should refer to 'adjustment with regard to some law, standard or reference ...', whereas 'control' describes in a more general sense: 'the exercise of restraint or direction upon'. The term 'regulator' might therefore imply a mode of action that was not applicable in all cases (but see section XI, 8).

Fig. 1 Potential complexity in plant hormone responses. The diagram is divided into four columns. A 'simple' model of action is provided in column three, while columns one and two indicate the location of events and some spatial issues. Possible complications are illustrated both in the diagram and are noted alongside each component in column four. For each of these, there is at least some evidence from published results.


Comments
Location
Simple model of action
Important elements to consider
Site of synthesis and response may not be separate
Site of synthesis
Stimulus perception
Anabolites
Catabolites
Pool of active moiety H
Active moiety itself may not be translocated
Trans-location system
Pool of active moiety H
Events below this can affect tissue sensitivity
Site of response
Anabolites
Catabolites
Receptor-hormone complex HRec
Receptor R
Responding cell
Second messenger(s)
Other influences affecting response
Interactions with membrane components and/or genome
Observable response may involve many combined cell responses
Responding tissue
Initiation of response
Environmental or ontogenetic stimulus?
De novo synthesis or from store?
Role of anabolism and catabolism
Site and size of pool of H
Compartmentation, storage, inactivation
Autocatalysis; feedback effects on synthesis
Release of H by synthesising cells/organelles
Is there a physical separation of sites of synthesis and response?
Does hormone transport occur?
Compartmentation, storage, inactivation close to active site
Metabolism and pool size
Concentration-dependent binding to receptor, probably on plasmalemma
In some cases, receptors may (also) be in cytoplasm
Roles of receptor synthesis and receptor isoforms
There may be a lag before response
Feedback processes are likely to operate, modifying initial response

At one point, the expression 'plant growth substances' seemed to have gained general acceptance (e.g. Audus, 1972). This, unfortunately, is a misnomer because the plant hormones and related substances do not always operate through the control of growth *per se*, or even through allied effects on differentiation or cell division. For instance, several examples have emerged of hormone-mediated responses to changes in the environment (for example, abscission, senescence, stomatal closure during water stress, as well as defence and wound responses), and therefore use of this term may be misleading. To reduce the phrase to 'plant substances' would clearly be absurd. Regarding compounds such as fusicoccin and kinetin not known to be naturally synthesized by higher plants, yet capable of affecting physiological processes at low concentration, there is at present, no adequate term.

Because the word hormone carries with it connotations of translocation, this might cause problems for its use in the plant case. To accommodate this problem, Mohr & Schopfer (1994) distinguished between compounds acting in the 'classic' hormone mode as 'transportable messenger substances' while those compounds where the site of synthesis and action were the same (whether response was induced by a change in concentration or a change in sensitivity) were termed 'autochthonous' (i.e. stationary) 'signal transmitters'.

The difference between 'hormones' and 'vitamins' is indistinct in some cases (see Audus, 1972; Morton, 1974). One approach is to separate the two on the basis of type of action (Phillips, 1971). Thus Folkers *et al.* (1970) defined a 'vitamin' as 'an organic substance of nutritional nature present in low concentration as a natural component of enzyme systems' which 'catalyses required reactions and may be derived externally to the tissues or by intrinsic biosynthesis'. Vitamins are thus, generally, enzyme cofactors, and whilst hormones may affect cellular proteins directly, this is probably in an allosteric fashion rather than at the site of action. The vitamin concept is more relevant to the heterotrophic animal and lower plant groups, since these must take in vitamins with food. However, aspects of the translocation of such cofactors as nicotinic acid, pyridoxine and thiamin between the shoot and roots of plants are hormonal (Thimann, 1948) as may be those of some nutrients (Trewavas, 1983c; Parsons *et al.*, 1993).

Ethylene is unusual in that it is a gaseous plant hormone, but that is not unique in biology. Nitric oxide is established as an important regulator in animal physiology, and probably will be shown to be as important in plant systems (Beligni & Lamattina, 2001). There are other volatile compounds apparently active in plant physiology, such as methyl jasmonate and methyl salicylate (Farmer & Ryan, 1990; Shulalev *et al.*, 1997). In the latter instance, these compounds, and ethylene, may qualify as 'pheromones', molecules that signal between individuals of the same species. Others may be 'allelochemicals', acting between species, although these are more frequently soluble compounds such as phenolics (Rice, 1984; Rizvi *et al.*, 1992).

Mer (1968) discussed the classification of inhibitory compounds, stating that a true 'inhibitor' inhibits a process normally occurring rather than one specifically promoted by another substance. For the latter case he suggested the term 'counteractant'. Larsen (1955) defined 'growth inhibitors' as 'substances which retard growth both in shoot and root cells and have no stimulatory range of concentrations'. Several authors consider the phrase 'inhibitory hormone' to be an oxymoron, since the word hormone is derived from the Greek 'to stimulate' (e.g. Phillips, 1971). However, this may depend on the precise way in which the response is defined: ABA may inhibit stomatal opening or stimulate stomatal closure; it may stimulate K^+ efflux from guard cells or inhibit H^+ uptake.

Whilst pedantic adoption of definitions often restricts practical usage, nomenclature in this area, as elsewhere in science, needs to take account of precedent (Huxley, 1935) and etymology (Larsen, 1955). It is clear that there is scope for much disagreement about nomenclature in the plant hormone field, and nearly all of the terms used so far have weaknesses. Perhaps this issue needs to be revisited by the plant physiology community.

In a keynote address to a conference convened to provide a critical appraisal of hormone action in plant development, one noted researcher summed up his feelings about the then current situation by stating: 'hormones are what physiologists call hormones' (Leopold, 1987). In this review, an open definition will be used, in part agreement with this philosophy, or at least the related one: 'plant hormones are what we eventually find them to be'. The terms 'plant hormone' and 'phytohormone' will be taken as synonyms to encompass any controlling influence that is an endogenous chemical (see also Weyers *et al.*, 1987, Weyers *et al.*, 1995). The question of transport between spatially separated sites of synthesis and action will be treated as a matter for investigation in each case. However, in adopting the term in this open sense, it is essential not to avoid issues about what constitutes control and how any candidate compound can be judged as a controller, as will be discussed below.

IV. The need for sound conceptual frameworks in plant hormone research

All scientific research takes place within a framework of understanding that underlies the hypotheses examined in any given series of experiments. Examples might include the principle of evolution by natural selection, or a particular taxonomic classification. Some concepts, such as the laws of physics governing gas exchange, are well-established, while others, like the cohesion-tension notion of xylem sap ascent, remain the subject of debate even after centuries of research (see Steudle, 2001). Clearly, from the discussion in sections II and III, the nature and role of the plant hormones falls into the latter category.

A lack of clear thought seems to have pervaded this research area, arguably due to the reluctance of plant hormone

physiologists to find and use an agreed conceptual framework to underpin research. This should be contrasted with the near-universal and successful use of Koch's postulates in the pathology of infectious diseases in both animals and plants. Without an explicit statement of the underlying rationale, it is difficult to design intelligent experiments to test hypotheses, and make progress in the field on a logical basis. Worse, as new evidence appears, there is a danger that old hypotheses (the 'classic' hormone one in this case) become adapted and extended *ad hoc*, without a clear picture emerging. A related point was made by Firn & Digby (1980) and Firn & Myers (1987), who noted that the history of research in the area of hormonal control of gravitropism is one of constantly modified hypotheses in response to 'unsupportive' evidence, rather than one of root-and-branch re-examination of results and theory.

A review of criteria for judging control by an endogenous chemical is conveniently introduced by considering the two basic types of evidence that have stimulated interest and provided the justification for work in this field.

The first is the observation of numerous physiological phenomena that can superficially be explained by the existence of a controlling chemical influence. There are many correlations between developmental events in plants, and these have frequently been explained by positing the existence of chemical messengers. The results of decapitating organs, introducing physical barriers or application of inhibitors are most conveniently interpreted in terms of the interruption of the supply of these hypothetical compounds. Also, certain mutations and genetically manipulated plants that possess altered hormone levels have phenotypes that support this analysis.

The after-effect of stress on stomatal movements (Davies *et al.*, 1981) is an example. This response indicates that a long-lived influence acts to keep stomata closed, even when the plant has regained turgor. This interpretation is supported by observations on a range of mutants that wilt readily and essentially cannot control their water relations via stomatal closure. Some of these mutants are deficient in the plant hormone ABA, which has been hypothesized as the controlling chemical influence. Theoretical considerations relating to the compartmentation of ABA can explain the effect, although further data are required (Hartung & Slovik, 1991).

The second consideration is the extraordinary efficacy of many endogenously occurring phytochemicals in eliciting plant responses. Examples of 'effectology', as it is sometimes disparagingly termed, are commonplace due to the ease of carrying out this type of research. Also, such studies have been underwritten by the agrochemical industry, which has at times collectively believed in the lucrative possibilities of wholesale manipulation of crops through the application of physiologically active compounds, or their use as weedkillers.

Of course, if a chemical had no effect on plants it would not even be considered as a potential controlling agent, and members of the five major hormone groups (ABA, auxin, cytokinins, ethylene and gibberellins) elicit an extraordinarily wide range of responses in plant systems (Scott, 1984). Continuing to use ABA as an example, this compound has been proposed to have roles in stomatal regulation, dormancy and abscission, as well as an involvement in responses to flooding, pathogens and wounding (Moore, 1989; Davies & Jones, 1991). Looking in detail at the stomatal response, it is extremely sensitive in the sense of requiring very small amounts of ABA (Ogunkanmi *et al.*, 1973; Weyers & Hillman, 1979a). It is highly selective, in that no other compound with similar structure gives rise to a similar response and even optical and geometrical isomers are ineffective in this system (Milborrow, 1974; Orton & Mansfield, 1974). The effects of ABA are extremely rapid, resulting in stomatal closure within minutes (Weyers & Hillman, 1979b); and it is effective on isolated guard cells and guard cell protoplasts (Schnabl *et al.*, 1978).

However, not all phytohormones are effective at such low doses (Trewavas, 1981). Although this may reflect the difficulty of penetration to active sites (see section VIII), there is possibly more than a grain of truth to the claim by Trewavas (1986b) that 'growth substances are often ascribed an importance which reflects only the ease with which the tissue can be treated with high doses of growth regulators'. From this standpoint, the ability of an endogenous chemical to elicit a response implies only the potential for it to be a controlling influence, but is by no means sufficient information in itself to make this a firm conclusion. For example, there would a requirement not only for the concentration of the endogenous compound at its presumed site of action to be within the physiologically active range but also for it to change sufficiently to give the response observed. In addition, this verdict would not take into account variable sensitivity (section VIII) nor the relative effects of alternative controlling influences over the phenomenon in question (section X). The phenomenon of allelopathy provides a salutory example where numerous alternative possibilities must be eliminated before chemical effects can be assumed (Williamson, 1990).

Similarly, there may be alternative explanations for the physiological phenomena that apparently involve chemical influences. The experimental manipulations often used could disrupt transport processes, disturb the water relations and nutrition of the tissues and may cause independent responses due to the wounding involved. They may also hypersensitize tissues to influences like plant hormones in an abnormal way (Trewavas, 1981). Nor is the existence of hormone mutants with physiological defects an absolute guarantee of the hormone's involvement. There are the possibilities of pleiotropy and of secondary effects to consider. Care is required to identify whether the mutation is one of hormone deficiency, hormone overproduction, hormone insensitivity or hormone hypersensitivity (Mohr & Schopfer, 1994).

The need for evidence beyond these two fundamental classes of observations led several researchers to create structured frameworks to accommodate evidence about hormone action. Table 1 summarizes the key papers and reviews dealing with

Table 1 Selected milestones in the development of the plant hormone concept

Author(s) and date(s) of publication	Key concepts discussed
Darwin & Darwin (1880)	Indications that a light stimulus perceived in the grass coleoptile apex can influence growth in lower parts
Bayliss & Starling (1902); Starling (1905)	Discovery of an animal chemical messenger and coining of the term hormone
Fitting (1909, 1910)	Discovery of a chemical message during fertilization-induced flower senescence: coining of the term phytohormone
Cholodny (1927)	Notion of differential organ sensitivity combined with lateral auxin transport in tropic responses
Thimann (1948)	Description of different sensitivities of organs (shoot, stem, root) to auxins via dose–response curves
Foster *et al*. (1952)	Kinetics of hormone responses described using Michaelis–Menten kinetics
Jacobs (1959)	The PESIGS rules described and applied to apical dominance
Drury (1969)	Notion of 'interactions' between plant hormones
Reeve & Crozier (1980)	Principles of rigorous chemical identification and quantification of hormones summarized
Cleland (1983)	Notion of combined control, shared between changes in hormone concentration and hormone sensitivity
Jackson (1987)	Re-analysis of the PESIGS rules: emphasis on quantitative, molecular and holistic aspects
Firn & Digby (1980); Digby & Firn (1981)	Emphasis on characterization of temporal and spatial aspects of response in tropic phenomena; criticism of modification of hormone concept *ad hoc*
Trewavas (1981)	Critique of simplistic notions of hormone action; emphasis on sensitivity changes; idea that physical and nutritional influences need to be taken into account
Cowan *et al*. (1982)	Idea that weak-acid hormones will be distributed in cellular and subcellular compartments according to local pH
Firn (1986); Weyers *et al.* (1987)	Recognition that sensitivity is not a simple unambiguous concept. Attempts to quantify sensitivity by fitting and comparing dose–response curves to Morgan-Mercer-Flodin model
Trewavas (1986b); Weyers *et al*. (1995); Paterson *et al.* (2001)	Introduction of the control coefficient concept to plant hormone work. Quantification of relative control potential in a stomatal system

these issues. This table demonstrates that the conceptual framework for plant hormone research has altered over the years and the following sections detail some of the major issues raised during this time.

V. Development of criteria for chemical control

Underlying the synthesis-transport-action phases of classical hormone action are two essential components, namely transport and chemical control via concentration changes. The first is meaningless unless the second takes place, while the second can occur without the first. The irreducible core concept is thus the notion of control involving a chemical component. The evidence required to demonstrate that hormone transport has occurred will be discussed later, but meantime the focus will be on chemical control as a concept. How can it be demonstrated convincingly that this is occurring?

Jacobs (1959) published an influential review dealing with the question 'what chemical normally controls a given developmental process?' He proposed that this question could best be answered by reference to a set of criteria in the form of six rules with the acronym PESIGS. These can be paraphrased as follows:

- Parallelism. This rule requires a change in the level of the supposed chemical controller in parallel with a change in some developmental phenomenon (response, more generally). It effectively requires a demonstration of concentration-dependent control at the active site. It is thus assumed that: the chemical can be measured accurately and precisely in some 'compartment' held to be a site of action; and that an appropriate indicator of developmental or response status can be measured;
- Excision. Removal of the source of the chemical (i.e. the organ or tissue producing it) should result in no response to a stimulus normally initiating one. This excision can be physical, as in removal of the root cap or parts of it in experiments on gravitropism (e.g. Wilkins, 1984) or genetic, as in the study of hormone-deficient mutants (Thomas & Grierson, 1987);
- Substitution. Addition of the chemical should substitute for the stimulus in inducing a response in otherwise untreated specimens (the 'effectology' referred to earlier). This would apply also to restoration of a normal phenotype in the case of a hormone-deficient mutant. It is implied that the concentrations used to elicit a response should be similar to those found *in vivo* at the appropriate site of action;
- Specificity. Efficacy should be restricted to a single or a few compounds. The 'special nature' of control is expected to involve only one substance. This is difficult to demonstrate where test compounds may be metabolically linked, as with the gibberellins (Phinney, 1984; Takahashi *et al.*, 1986). This notion was to develop into the related concepts of sensitivity and control strength (in that the response should be more sensitive to the presumed controlling compound than to other influences – see sections IX and XI);

• Isolation. It should be possible to demonstrate that the chemical-response relationship is maintained in experimental systems separated from the whole organism. The idea here is to reduce the possibility that a completely different controlling system is involved. Of course, this approach carries with it the risk of causing damage and wounding effects in the isolation process;
• Generality. This criterion states that the same system should be found in a several species. This is assumed to provide greater confidence in the hypothesis. Ideally, perhaps one would be able to see trends in an evolutionary sense, with compounds, receptors and response systems being modified for subtly different purposes in related species.

An important feature of these rules is that no one criterion alone provides sufficient evidence to indicate that chemical control might be occurring. For example, parallelism could easily occur by coincidence or because the observed phenomena and changes in hormone levels were simultaneously controlled by another unknown event. By providing evidence in most if not all categories, researchers can tip the balance of probabilities in favour of the hormone thesis. Jacobs tested the rules on the suggested involvement of auxin in apical dominance, and concluded that the IAA might be the correlative influence in this phenomenon.

Jackson (1987) provided the next substantial review of criteria for judging the hormone-like nature of a compound. His proposals for a structured evaluation refined those of Jacobs (1959) taking into account the need to link studies at a subcellular or molecular level with those at higher levels of organization such as tissues, organs and plants, and in turn to ecological and evolutionary aspects. This version also moved towards a quantitative approach to the problem. Jackson's rules can be summarized as follows:
• Correlation. This is the same as Jacobs' 'parallelism', but with emphasis on the precision of timing. Simultaneous changes in the timing of developmental change and in hormone content should be observed, the ideal being precedence in hormone levels commensurate with the known speed of action. Zucconi (1988) made the interesting point that this is not necessarily a requirement in complex integrated systems: he provided an illustrative model in which effect can precede apparent cause, given assumptions that are feasible, if unlikely;
• Duplication. It should be possible to reproduce the physiological phenomenon by manipulation of the internal hormone concentration, duplicating, as far as possible, the endogenous changes that occur naturally. This is the same as Jacobs' 'substitution', but with emphasis on mimicry of effects at quantitatively similar values;
• Deletion and re-instatement. This criterion can be satisfied, firstly, by showing that removing or decreasing the endogenous hormone titre results in prevention or inhibition of the physiological response, and, secondly, by reversing these effects by reinstating the endogenous hormone levels artificially (demonstrably to the natural concentrations). Competitive inhibition of a hormone's effects, preferably reversibly and without toxic effects, is admissible evidence under this category. This rule is a combination of Jacobs' 'excision' and 'substitution' categories, with a quantitative element;
• Chemical specificity. The evidence in the preceding categories should not be found for other substances, other than precursors of the single active compound. As the name implies, this is essentially the same as Jacobs' 'specificity' criterion;
• Relevance to higher levels of organization. This rule indicates that the process studied should be observable outwith the 'confines of the laboratory'. It should relate quantitatively to performance in environments to which organs, whole plants or populations of plants are naturally subject. This is a new and important rule that ensures realism in experiments. This rule takes into account the danger that the extreme control evident in experimental conditions may be inadvertently manipulated to increase tissue sensitivity to a compound, or to exclude consideration of the response to other stimuli in favour of the putative hormone (Weyers *et al.*, 1995). These aspects will be discussed further when the role of hormones in the context of other environmental variables (Trewavas, 1986b) is covered in section X;
• Relevance to lower levels of organization. Here, it should be possible to demonstrate that the association between exogenous hormone and response is retained at cellular, subcellular and biochemical levels. Essentially, confidence in a hypothesis framed at the tissue level is seen as strengthened, the more molecular detail that can be attached to it. For example, if a hypothetical hormone receptor can be identified in the tissue under question, and if its binding properties quantitatively match our expectations from other measurements, then this allows the hypothesis underlying the initial notion to be maintained, rather than rejected. This is a development of Jacobs' 'isolation' category. In Jacobs' original sense, by teasing out the details of a realistic response in successively reduced parts of the plant, the possibility of other control elements being involved can be ruled out, or taken into account;
• Generality. This criterion states that the same response system should also be found in related taxonomic groups. This is the same as Jacobs' rule of the same name, invoking phylogeny as a key to understanding of the evolution of the response.

While the first four and the last of Jackson's seven categories are essentially the same as five of Jacobs', Jackson (1987) emphasized quantitative aspects and molecular detail. This was presumably aimed at countering criticisms that much of the previous hormone literature tended to take little account of response timing, used exogenous compounds at concentrations vastly in excess of those known to prevail at the tissue level and/or assumed that small changes in concentration could elicit large responses (Trewavas, 1981). The fifth and sixth categories of Jackson develop one category of Jacobs (1959), promoting an analysis of the phenomenon at organizational levels above and below that being studied and forcing the response to be seen in its full organismal and evolutionary context.

Jackson (1987) demonstrated his approach by successfully applying these criteria to the action of ethylene and abscisic acid in flood responses by tomatoes and peas. In so doing, he used his analysis to pinpoint where future research might usefully be directed in these areas. However, neither Jacob's PESIGS rules nor Jackson's modification to them are frequently referred to in the literature. Researchers (and journal editors and referees) seem to be content that each paper on a hormone response is considered in isolation as a small step towards a greater understanding, perhaps later to be reviewed as such. Arguably, greater progress might be made if authors took it upon themselves or were asked to indicate where in such a framework their evidence fitted.

On the other hand, overly pedantic operation of rules can be counter-productive. Chemical control can be envisaged to exist even though certain of the rules might be broken. For example, one would not perhaps wish to exclude a compound as a chemical controller solely because no other species than the one tested had been found to have the same system (Jacobs' generality criterion). This could be due to be a new evolutionary development or (more likely perhaps) because of ignorance on our part. However, the rule violation ought to be discussed as such. An example of just such an evolutionary innovation in plants can be seen in the polypeptide defence hormones, where systemin is found in several closely related members of the Solanaceae, but not in other representatives of that family (Ryan & Pearce, 2001).

The next significant development came with the incorporation of sensitivity into research frameworks. Trewavas (1982) had made the logically correct point that a response could actually occur if there were solely a change in tissue sensitivity, and no change in concentration, and in fact put this forward as a primary mode of action of plant hormones. Firn (1986) and Weyers *et al.* (1987, 1995) concluded that the only way in which changes in tissue sensitivity could be investigated was via changes in dose–response (D-R) curves and the latter authors proposed statistically valid methods for quantifying and comparing such curves.

Cleland (1983), Salisbury *et al.* (1988) and Weyers *et al.* (1995) noted the three logical possibilities for control over the observed response (illustrated in Fig. 2):

1 Chemical control, where the D-R curve might not change throughout the period considered, but the hormone concentration ([H]) eliciting the response would alter, leading to a different response (Fig. 2a). This is compatible with the classic view of hormone action where the transport system delivers a higher [H] to the responding tissue, but it does not necessarily require transport to occur;

2 Control via sensitivity, where [H] might not change throughout the period considered but the tissue sensitivity would alter (as indicated by changes in the shape or position of the D-R curve, Fig. 2b). Thus the response would be controlled solely by tissue sensitivity to a constant hormone pool. This is Trewavas' (1981) 'extreme opposite position' to the classic view of hormone action (Cleland, 1983);

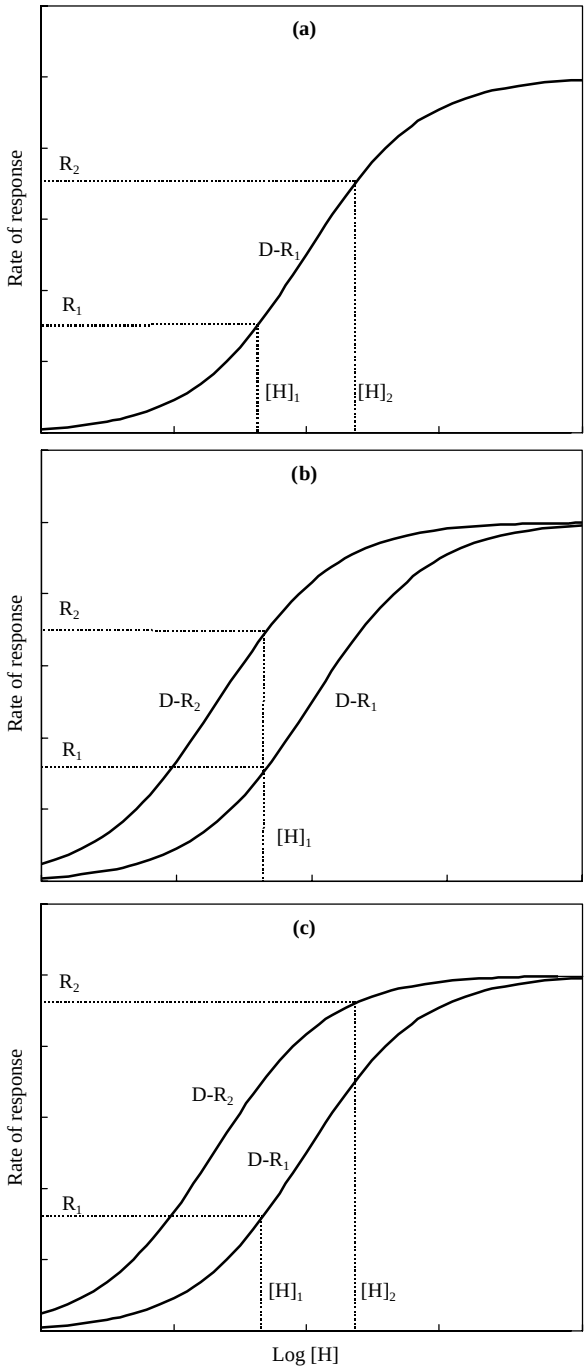


Fig. 2 Illustration of three ways in which a response could be controlled (after Weyers *et al.*, 1995). (a) Chemical control: the dose–response curve (D-R1) is unchanging and the response rate alters from R_1 to R_2 due to a change in hormone concentration at the site of action from $[H]_1$ to $[H]_2$. (b) Control via sensitivity: the hormone concentration is unchanging at $[H]_1$ and the response rate alters from R_1 to R_2 because of a change in the D-R curve from DR_1 to DR_2. (c) Combined control: both [H] *and* the D-R curve change and response alters from R_1 to R_2, a greater change than in the other two cases which involve the same change in H and D-*R*-values, but operating in isolation. Note that in this diagram, the sensitivity change is modelled as a shift in H_{50} but there are other ways in which the dose–response curve could alter that would also affect the response (Weyers *et al.*, 1987).

3 Combined control, where both [H] and the D-R relationship might alter during the period considered (Fig. 2c), meaning that control over the observed response would be shared between hormone levels and tissue sensitivity. This was considered by Cleland (1983) and Weyers *et al.* (1995) to be the condition that should be assumed in the absence of contrary information.

Recognizing that control over response could be exerted from both the synthesis/transport and response elements of the system, Weyers *et al.* (1995) produced a simplified three-criterion framework for the involvement of a hormone in a response. This emphasized the importance of D-R curves for rigorous quantitative definitions within the spirit of the Jacobs–Jackson criteria and introduced a new concept, that of 'relative control potential', as quantified via 'control coefficients' (see section X). The Weyers *et al.* (1995) rules were stated as follows:

- There should be a quantitative match between observed hormone concentration changes and responses predicted from D-R curves. In the spirit of combined control, changes in D-R curves should be taken into account, as an indicator of variation in sensitivity, as well as changes in [H]. The predicted responses generated by measured values of [H] and the relevant D-R curves should be quantitatively consistent with the measured rates of response *in vivo*, it being assumed that conditions are equivalent. Any changes in concentration or sensitivity should occur on a timescale commensurate with the observed changes in response *in vivo*. This criterion combines the 'parallelism' and 'substitution' criteria of Jacobs ('correlation' and 'duplication' in Jackson's framework), but also allows for the possibility of combined control.
- Manipulation of [H] or of sensitivity should have the predicted effect on responses. The manipulation can be achieved via genetics, inhibitors, substitution (*sensu* Jacobs, 1959), or other treatments. It should generate changes in response rates comparable to those predicted from measurements of the change in [H] and the appropriate D-R curves. Where possible, reinstatement of the natural concentration should result in a return to a natural response rate. This is based on the 'excision' and 'substitution' criteria of Jacobs and the 'deletion and reinstatement' rule of Jackson, but allows for experimental changes in sensitivity and the possibility of combined control.
- The control coefficients for H should be relatively high. This novel criterion is a means of determining how important a given factor might be in determining a response via combined control in the context of the system's potential responses to other influences, whether be they to other hormones, chemicals or physical factors. Methods for quantifying control coefficients are discussed in section X. Control coefficients of all relevant stimuli at a relevant set of conditions should be compared and 'normal' ranges of values for stimuli should be taken into account in arriving at an assessment of their effectiveness. The relative control potential for the compound of interest must be relatively high if it is to exert control.

To adopt the framework of Weyers *et al.* (1995) successfully, it was regarded as essential that D-R curves are obtained in an appropriate manner; also, the relevance of conclusions was seen to depend critically on the accuracy of estimates of [H] at its site of action. It was noted that both these stipulations require extreme care in experimental design and methodology. Weyers and coworkers have applied their criteria to the role of ABA in control of stomatal movements during water deficit stress (Paterson *et al.*, 1990; Weyers & Peng, 1995; Weyers *et al.*, 1995).

VI. Identification and quantitative analysis of plant hormones

As noted above, the analysis of hormone concentrations in tissues and particularly at putative sites of action is crucially important in establishing a chemical's potential role in a given phenomenon. There are two main issues at stake: first, reliable identification and quantification techniques for any assay of hormone concentration; and second, establishment of the precise site of action and estimation of the hormone's concentration within this compartment or pool.

Proper analysis of plant hormone levels occupied much journal space between 1960 and 1980. Debate centred on the fact that bioassays, as used in much research before 1960, did not provide a molecular identification of any chemical, an absolute prerequisite for quantification (Reeve & Crozier, 1980). This was not the only uncertainty with bioassays, because other problems related to linearity, sensitivity and reproducibility of response were also uncovered. Consequently, all the research that had relied on bioassay determinations was called into question. It should be noted, however, that the D-R relationships used in bioassays are essential for determining potential physiological activity and for quantifying sensitivity.

It became evident that complex techniques such as mass spectroscopy and infra-red spectroscopy were required to provide the required chemical information at appropriate levels of sensitivity and selectivity (Hillman, 1978; Reeve & Crozier, 1980). These methods involve costly apparatus and their successful application requires a high degree of technical knowledge. The associated need for high purity samples for analysis also necessitates expensive equipment and a high level of expertise.

For plant extracts, special difficulties are associated with the highly complex mix of molecules present in unpurified samples and the low concentrations at which the compounds of interest are to be found in these mixtures. Important considerations include quantifying the inevitable losses of material when purifying – difficult but essential for accurate measurement. Methods that involve spiking samples with isotopically labelled standards have been developed and tested successfully. Although it is now technically possible to achieve reliable estimates of hormone contents of plant tissues it must be said that relatively few laboratories are able to make such determinations

on a routine basis. Radioimmunoassay (essentially a form of bioassay) has emerged as a more convenient method (Weiler *et al.*, 1986), although aspects such as selectivity and interference from impurities need to be taken into account.

Despite the application of advanced chemical methods to the problem, and the breakthroughs in methods and understanding noted above, fundamental questions remain unanswered. The focus on accurate quantification effectively deflected attention from issues such as what precisely should be measured, and where and when, and how measurements should be expressed. Fundamental physiological problems need to be addressed before it is appropriate to expend resources on hormone measurements. Precise details of the time and place of response are required before appropriate measurements of a plant hormone can be made. Moreover, the precise site of action is likely to be subcellular, close to the primary receptors for the hormone signal. Two problems then emerge – the first being to find the identity and location of the receptors (see Section IX). The second is measuring the hormone concentration in such small volumes.

Hormone compartmentation within cells and tissues has long been recognized and has been given as a possible explanation as to why whole tissue or organ hormone concentrations do not exhibit 'parallelism' in the sense of Jacobs (1959). While little quantitative information has been available at these scales, novel techniques may shed light on the problem (Trewavas, 1991; Zhang & Outlaw, 2001). Meanwhile, a partial solution has been provided by Cowan *et al.* (1982), and developed further by Hartung and coworkers (Hartung & Slovik, 1991). For the major hormones, Hartung & Slovik summarized a range of parameters related to pH and membrane permeability. They noted that ABA, being a weak acid and effectively only permeable to membranes in the undissociated form, would distribute itself across cell membranes according to the pH and volumes of the relevant compartments. This meant that a whole tissue measurement of the hormone could lead to a prediction of the concentration of the hormone in different compartments if the pH and volume of the compartments were known (Cowan *et al.*, 1982). Weyers & Paterson (1992) applied this type of analysis to the distribution of ABA during water stress. Experiments reveal that receptors for IAA and ABA lie on the outside of the plasmalemma (Hartung, 1983; Venis *et al.*, 1990; Allan & Trewavas, 1994), which implies that, at least in certain cases, the apoplasm may be an important compartment. Unfortunately, because of differences in their characteristics, other acidic hormones such as IAA and gibberellins are less amenable to this method (Hartung & Slovik, 1991). Recently, however, Zhang & Outlaw (2001) succeeded in using a combination of microdissection, differential wash-out and immunoassay methods to follow ABA concentrations in the guard cell symplast and apoplast during a stress treatment, allowing valuable correlations to be made between stomatal responses and ABA levels at the putative site of action.

The rate of metabolism is another important determinant of hormone pool size and hence concentration. In some systems, the relevant changes in rates of anabolism and catabolism may control the active pool size on a minute to minute basis (Hartung *et al.*, 1998). This presupposes that a single physiologically active moiety can be identified – not easy to determine when there may be many different precursors and catabolites present in tissues, which may themselves may also have residual activity. A good case in question is the gibberellins, the number of which rose from one in 1954 (Curtis & Cross, 1954) to over 125 by 2000 (Crozier *et al.*, 2000). With the aim of establishing the metabolic pathways, a great deal of effort went into the chemical identification of gibberellins. There has been relatively less work on the fluxes through metabolic pathways, pool sizes, subcellular location and other information required to establish what might be the active compounds. Different gibberellins have been shown to be active in different systems (Phinney, 1984), so the concept of a pool of a 'physiologically active' gibberellin within each tissue has become accepted, with other compounds being regarded as metabolites (Fig. 1).

Matters were complicated by the discovery of various conjugated ('bound') derivatives of the plant hormones. Examples of such conjugates for ABA, auxins, cytokinins and gibberellins are given in Sembdner *et al.* (1980). These could be viewed as 'detoxified/inactivated' compound or as a metabolically compartmentalized pool of compound ready for rapid mobilization when required (by stimulation of hydrolase activity, for instance).

Some authors have sought to explain complexities of the relationship between endogenous hormone levels and phenomena by the notion of hormone 'interactions' (Drury, 1969). This term is generally not used in its statistical sense to involve antagonism or synergy *sensu stricto*, with the combined effect, measured on a linear scale, being less than or more than the arithmetic sum of the individual effects. Rather it is used to indicate that a balance or interplay between hormone concentrations might control events. A leading proponent of this view was Wareing (1977), who gave examples where application of different hormones gave rise to opposite responses in assays, or when compounds were applied in different ratios, they elicited different responses. There were also cases where hormone levels altered together or in opposite sense to each other. Wareing (1977) stated that frameworks like those proposed by Jacobs and others were not applicable in a situation where control appeared to involve an interaction between the effects of two or more compounds, although the list of types of evidence cited above immediately suggests some criteria. For a long time, there was little quantitative or molecular evidence to support the idea of hormone interactions at the molecular level. However, several recent reports demonstrate that specific plant hormones are able to influence the biosynthesis of another or interfere in the signal transduction of another (McCourt, 1999; O'Donnell *et al.*, 2001; Ross &

O'Neill, 2001). These findings allow the formulation of quite specific hypotheses, testable within a framework similar to those described in section V.

VII. Hormone transport in plants

None of the criteria for establishing chemical control mentioned above include a rule that assumes spatial separation of sites of synthesis and response, nor do they suggest a framework for establishing whether a proposed hormone is translocated within a plant. However, if one wished to investigate this possibility, or attempt to demonstrate the 'classic' type of hormone action, then such evidence would be required. In operating any set of criteria for this, one must recognize certain key features of plants.

First, the internal organization of plants renders the organ-to-organ control of classical mammalian hormones a less useful concept. This is overly restrictive since anatomists consider there to be only four organs in plants: stem, leaf, root and reproductive structures (Esau, 1965). From both morphological and functional considerations, it is more suitable to divide plants into tissues, and where translocation of hormones is to be considered, to consider movement between such tissues (in fact, this modification is also required for some animal hormones). A complicating factor is the nature of differentiation in plant tissues compared with that between mammalian organs. Transitions between plant tissues are far less 'discrete' in nature, resulting in cells that adopt hybrid forms (and presumably hybrid physiology). There are consequent difficulties in determining the physical (and physiological) boundaries between tissues and physically isolating the component parts of the system.

The distance between sites of synthesis and action could vary from that between organs to that between cells. This encompasses a 1 000 000-fold difference in scales, from distances of metres to those measured in μm. In this review, intracellular messengers such as mRNA, enzyme effectors and second messengers such as cAMP and Ca^{2+} will not be considered as phytohormones, although there is an argument that they are part of a continuum of chemical controllers exerting influence from distances between molecules to those between organs, and even between organisms in the case of the pheromones (Huxley, 1935).

Second, there is the need to identify the translocation systems that are possible and to take account of their mode of operation. Despite the inherent difficulties in sampling from the xylem and phloem (section II), there is a need to consider the logical restrictions arising from flux control and direction in these systems. Thus, delivery via the xylem is primarily to sites of transpiration, where dissolved substances will inevitably be concentrated as the water solvent evaporates unless they are compartmentalized or metabolized in the mesophyll (Trejo *et al.*, 1995). How effective can an internal control system be when the delivery of the controlling agent is capriciously affected by external events, such as windspeed and humidity? In the case of the phloem, translocation is controlled by source and sink activity, but insofar as the rates of these processes may be affected by irradiance, temperature and water relations (Nobel, 1991), delivery might also be affected by external factors (Peuke *et al.*, 2001). In fact, hormones have been found in the saps of these systems, so logically they will be delivered at rates determined by outside factors. Turning this argument on its head, might they have an important role in integrating activity in response to such changes?

Although found in the xylem and phloem sap (King, 1976), IAA is also transported via a specialized polar transport system that differs in several respects from conduit-based movement: it is unidirectional, from apex to base; its speed is slower, being similar to that of diffusion; it can operate against a concentration gradient; and it is specific for IAA (Canny, 1984). Studies with *Arabidopsis* mutants have allowed the characterization of putative auxin influx and efflux carriers involved in the putative mechanism for tropic behaviour (Swarup *et al.*, 2000).

While acknowledging the existence of several possible transport mechanisms (with associated interactions and other complications, as discussed above), the hormonal 'action at a distance' concept still requires demonstration of transfer of a compound between tissues, and it may be desirable to test this in some cases. How can this be done? Through analysis of past research and reviews on this topic (Ziegler, 1975; King, 1976; Jackson, 1993), the following set of criteria can be put forward:

- The compound should occur in the translocation system at appropriate concentrations. It should be possible to show this by identifying the compound of interest within transported sap. Additionally, this should be at a concentration that quantitatively matches the sensitivity of the tissue to which it will be delivered; in other words, at values that might be expected to influence response. Assuming a classical hormone hypothesis (i.e. chemical control), then changes in concentration should occur in sufficient amount and with appropriate timing. Furthermore, if the proposed source of the compound is 'excised' as discussed in the Jacobs–Jackson criteria, then levels in the translocation medium should fall at a rate commensurate with the loss of response. While samples of xylem and phloem sap can be obtained, the act of their isolation using current techniques is likely to so disrupt the functioning of the translocation system that it might be extremely difficult to determine the dynamics of endogenous hormone movement with any reliability. In the case of the symplastic transport system, the relevance and realism of, for example collection on agar blocks, as traditionally used, must be called into question, even though no alternative seems to exist. Obviously, the criteria used to identify and quantify the levels of plant hormones in the transport fluids or 'diffusates' should be as rigorous as for other plant extracts (see section VI). In practice, the general lack of cellular contents in these saps confers an advantage because extracts may not need such extensive purification as those from whole tissues, and compartmentation is not an issue;

• Applied compounds should move in the proposed translocation medium at appropriate rates and directions. It should be possible to demonstrate that exogenous compound can be transported between known or hypothetical sites of synthesis and action at rates compatible with the signal-response timing normally found. The system should also have the capacity to translocate the amounts required to elicit action in the responding tissue, and with the appropriate polarity or direction. Demonstrating this has generally involved using compound labelled with some form of tracer. Much use has been made of compounds labelled with radioactive isotopes, but it is important to analyse the state of the labelled compound after transport, and account must be made for isotope discrimination effects. There are problems associated with donation to the relevant translocation system, as intervention may lead to loss of function. It might also be possible to rule out some translocation mechanisms *a priori*, because of their known limitations of rate. Thus polar auxin transport occurs at a maximum velocity of 0.015 m h^{-1} (Goldsmith, 1969), phloem transport occurs at 0.2–1 m h^{-1} and xylem transport at up to 3.6 m h^{-1} (Nobel, 1991). Diffusion (relevant in the case of ethylene, for example) can be described by Fick's laws (Nobel, 1991). It should be noted that ethylene is actually more soluble in water than most other hormones, with a saturation concentration of over 4 mmol l^{-1};

• Interruption of the translocation system should affect the response as predicted. This has been a long-standing method and is, effectively, similar to the 'excision' and 'deletion and reinstatement' categories noted in section V. The use of physical barriers to interrupt transport has been extremely influential in research on tropisms. In a series of experiments that involved for the large part insertion of mica barriers into *Zea mays* primary roots, the respective research groups of M. B. Wilkins and P-E. Pilet were able to determine much about the mode of action of the putative root cap inhibitor (Wilkins, 1984). Physical barriers are not the only method applicable – tri-iodo benzoic acid (TIBA), an inhibitor of polar auxin transport, has been widely used, for example. Clearly there are questions to be answered about the wounding effects of barriers and the specificity of inhibitors, so this type of evidence needs to be carefully analysed and cross-checked. In many examples, difficulty in isolating the sites of synthesis and effect, combined with a dearth of knowledge about metabolite identities, have meant that it is difficult to identify whether a specific chemical is transported itself or as a metabolite (Fig. 1). In some cases, hormone conjugates have been hypothesized as the transported moieties (Wareing, 1977). Some gibberellins are transported and some not (Jones, 1973), while some are physiologically active and others not (Phinney, 1984) – it is theoretically feasible for a precursor of an active gibberellin to be translocated and converted into an active compound at or near to the site of action (Fig. 1). It would not be obvious in these cases which molecule might have true hormone status, the transported compound or the one which is active in the responding tissue. A further and significant difficulty is calculating the concentration of hormone at the end of the translocation system and thence at its site of action, should these be different (Jackson, 1993; Hartung *et al.*, 1998).

Trewavas (1986a) quoted several texts which explicitly cited transport as an integral part of hormone action. For example, Jacobs (1979) stated 'hormones are substances active in minute amounts produced in one location and active in another. In angiosperms, this movement coordinates development'; and Letham *et al.* (1978) wrote 'Phytohormones move from a site of production to a site of action to evoke characteristic responses'. The main reason for assuming that translocation might not always be required in the case of phytohormones is the general lack of a tissue-based locus of synthesis (as in the gibberellins) and the consequent presence of certain hormones in all parts of the plant at similar concentrations (at least on a whole tissue basis). However, Trewavas (1986b) also gave theoretical reasons why this form of control might not benefit plants or would not fit with observed behaviour. As will be stated in section XI, some putative roles for hormones require transport, while others do not; consequently, the need to apply the above criteria will depend on the phenomenon under study.

VIII. Hormone sensitivity and its quantification

'Response' to a chemical signal implies sensing by the responding cells. The notion of 'competence' to respond is attractive in plants as it is frequently found that groups of cells respond to hormone application rather than whole organs. This concept of 'tissue sensitivity' can encompass both spatial and temporal changes in the ability to respond to a stimulus. In fact, this has been appreciated for some time. For example, Went & Thimann (1937) make a number of references to the sensitivity of monocot coleoptiles to auxins. They generally appear to mean the term in the sense of 'threshold concentration', that is, the smallest concentration that elicits a significant response (Weyers *et al.*, 1987). Went & Thimann refer to their own and others' research indicating differences in sensitivity in this sense caused by irradiance, water relations and timing of treatment. They also comment on species differences in sensitivity, as reported by Söding.

Sensitivity differences between organs were an integral part of the Chlolodny-Went hypothesis of tropistic control. This theory, as applied to gravitropism, holds that lateral distribution of auxin within organs is influenced by gravity, with more appearing on the lower side. The differential sensitivity of organs to auxin is said to explain why roots grow down and shoots upward. This was neatly summarized by Thimann (1948), who compared auxin-growth D-R curves for roots, buds and stems. These were presented on a log-lin axis and were bell-shaped. At low concentration ranges, increasing auxin concentration caused an increase in growth relative to controls, whereas, after an optimum was reached, any further increase auxin concentration then caused decreasing growth,

with values eventually dipping below that of controls. The curve for roots was positioned over a lower concentration range compared with that for buds, differing by three orders of magnitude. In turn, the curve for buds was positioned over a lower concentration range compared to that for stems, differing by a further four orders of magnitude.

Thimann's analysis clearly indicated that the responses of the different organs to any specified auxin concentration might be completely different, and the response to a change in concentration might be positive or negative with respect to gravity, depending on the absolute concentration. Audus (1969) provided a figure that linked the likely changes in auxin concentrations with the dose–response curves for roots and coleoptiles, and could thereby explain the responses observed. Unfortunately, this did not take into account the evidence that a root-cap inhibitor different from auxin is likely to be involved (Wilkins, 1984). Nor did it consider reports of changes in the sensitivity of upper and lower parts of organs to IAA following a change in orientation with respect to gravity (MacDonald & Hart, 1987; Salisbury *et al.*, 1988). This body of work indicates that 'combined control' might well operate in gravitropism, although the subject is still controversial (see section II).

It was also evident that spatial and temporal differences in responses might be indicators of developmental changes in sensitivity (Trewavas, 1981). Osborne (1977) introduced the 'target cell' concept to indicate the differential sensitivity of certain cells to ethylene, and, in this sense, there are many other examples of cells with heightened sensitivity compared to their neighbours (e.g. guard cells and ABA). There are also examples of spatial and temporal gradients in the ability to respond to hormones (e.g. *Triticum* coleoptile growth and IAA – Wright (1966); *Spirodelia* turion formation and ABA – Trewavas (1981)).

Despite all of this work, there was no unifying theory behind the sensitivity concept. One of the main problems with giving sensitivity a major role in plant responses to hormones was that it appeared a rather nebulous term, being capable of multiple interpretations (Firn, 1986; Weyers *et al.*, 1987). It was seen as difficult to quantify and hence difficult to compare between different situations. The most obvious approach to answering this problem involves analysis of D-R relationships, since this is the only way of determining a tissue's ability to respond to a chemical stimulus. These studies start from the premise that some concentration-dependent reaction sequence elicits a response to the chemical influence, via reversible binding to a receptor. At the site of action, the process can be summarized as follows (Clark, 1933):

$$H + Rec \leftrightarrow HRec \rightarrow \rightarrow Response \qquad \text{Eqn 1}$$

(H, hormone molecule; and Rec, receptor.) From this relationship it can be seen that the response depends not only on [H], but also on [Rec], on the dissociation constant of HRec, and on the processes, which are likely to be many, that lead from the formation of HRec to the measured response. Seen in the context of this relationship, changes in sensitivity could arise from modifications to the properties or concentration of Rec, or the tissue's reaction to the formation of HRec.

Equation (1) is clearly analogous in many features to the situation in enzyme kinetics. Given certain assumptions about the nature and binding of hormone molecules to putative receptors, and about the manner in which the hormone-receptor complex is transduced to a response, the kinetics of hormone responses would therefore be predicted to fit the Michaelis–Menten equation. The expectation would be for a hyperbolic D-R curve in linear plots, and a rotationally symmetrical sigmoidal curve in log-lin plots.

Bonner and colleagues were the first to apply the Michaelis–Menten equation to describe the rate of *Avena* coleoptile growth in responses to auxin (Foster *et al.*, 1952; Bonner, 1954). This approach was not widely accepted at the time, largely due to its failure to explain the optima present in many auxin D-R curves (Bennet-Clark, 1956; see discussion below). It would also be problematic with observations of many other hormone D-R relationships, which although frequently sigmoidal in shape in log-lin plots, rarely fit this relationship, being either 'supersensitive' or 'subsensitive' (Nissen, 1985, 1988a, 1988b). In these cases, the change in response occurs over a narrower or wider range than would be predicted (see below). Weyers *et al.* (1987, 1995) pointed out that the four-parameter Morgan-Mercer-Flodin equation (Morgan *et al.*, 1975), an extension of the three-parameter Michaelis–Menten equation, might prove a better fit to these types of curve.

In the view of Weyers *et al.* (1987), the problem of describing hormone sensitivity (and how it may change) reduced to:
1 Finding a satisfactory mathematical model which can be fitted to real data carrying error.
2 Verifying statistically that there is a difference among fitted curves.
3 Attributing the differences observed to changes in specific parameters (while accepting that it would not be appropriate to assign physico-chemical meaning to the parameters in terms, for example, of receptor properties – see section IX).

Accordingly, and with important provisos, they developed ways of fitting (nonlinear regression) and comparing (step-down ANOVA) the following function to real D-R curves:

$$R = R_{MIN} + R_{AMP}\frac{[H]^p}{[H]_{50}^p + [H]^p} \qquad \text{Eqn 2}$$

([H], the hormone concentration; R, initial rate of response; R_{MIN}, minimum initial rate of response; R_{AMP}, difference between the maximum initial rate of response and R_{MIN}; p, dimensionless number; and $[H]_{50}$, value of [H] at $R = R_{MIN} + 0.5\ R_{AMP}$.) The use of initial rates of response was proposed to minimize complications due to the intervention of feedback mechanisms at later stages in the development of a response (see below).

From such analyses, it is evident that 'sensitivity' cannot be a simple unambiguous concept (Firn, 1986; Weyers *et al.*, 1987; Paterson *et al.*, 1990). A change in sensitivity will be evident in changes in the D-R curve and hence in one or more of the parameters of Eqn 1 and others derived from them (Fig. 3). Thus, although the term 'sensitivity' can have a variety of meanings, depending on the precise aspect of the D-R relationship under consideration, all of these can be expressed and quantified within this model (Paterson *et al.*, 1990). The precise sense in which it is being used should be defined in each case. Methods for describing and comparing D-R curves modelled by Eqn 2 were developed by A'Brook (1989) and are described in Weyers *et al.* (1987).

The parameters R_{MIN} and R_{AMP} allow for the possibility that the response in the absence of exogenous hormone can be nonzero. Nonzero values of R_{MIN} might arise due to: the effects of endogenous compounds; inherent random error; responses to disturbance; and/or the effects of other environmental stimuli. R_{AMP} is a convenient measure to describe additional responses above the R_{MIN} baseline, assuming such effects occur independently of the hormone response. Haga & Uino (1998) presented a modified form of Eqn 2 which took the possible contribution of endogenous hormone explicitly into account. This required the assumption that growth without an external supply of hormone was entirely due to endogenous compound (IAA in their case).

The model of Weyers *et al.* (1987) involves p, the so-called 'interaction coefficient', comparable to the Hill coefficient used in enzyme kinetics, which is a power to which [H] and $[H]_{50}$ are raised. As noted above, this allows for responses that do not follow Michaelis–Menten kinetics in terms of the range of [H] over which the effective change in response occurs (see Fig. 3). If $p = 1$ (and $R_{MIN} = 0$), then the model simplifies to the Michaleis–Menten equation; if $p > 1$, then the response is 'hypersensitive' and occurs over a narrower [H] range than defined by Eqn 2; and if $p < 1$, then the response is 'subsensitive' and occurs over a wider [H] range than defined by Eqn 2. The parameter p therefore represents a different, but mathematically related, method of describing differences in the 'sensitivity coefficient' (i.e. H_{90}/H_{10}) of Koshland *et al.* (1982) as invoked by Nissen (1985).

In constructing dose–response curves to investigate sensitivity, ideally the 'dose' (i.e. [H]) would be manipulated internally via control over synthesis/degradation of the compound of interest, perhaps via genetic engineering. In practice, the main way of adjusting [H] has been to apply exogenous compounds. The assumption is often made that the applied concentration is the same as that in the 'biophase' where it acts. However, this is unlikely for all but the most isolated of systems (Weyers *et al.*, 1987): for example, MacDowall & Sirois (1977) demonstrated that the extension rate of *Triticum* coleoptiles was limited by the rate of penetration of IAA into the tissues. Factors like metabolism and pH-related compartmentation might also alter the biophase concentration

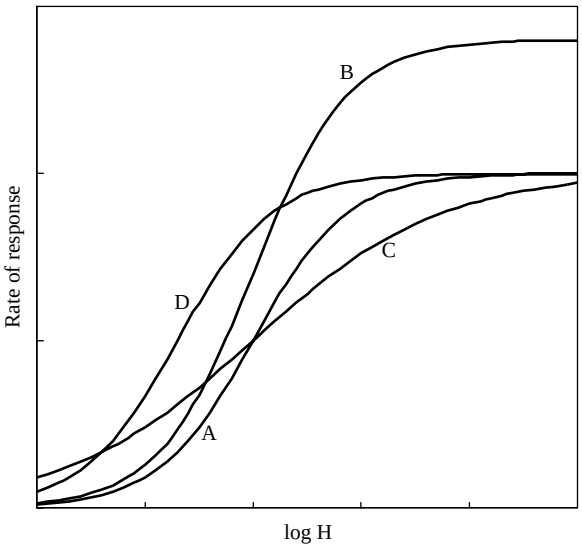


Fig. 3 Different possibilities for changes in sensitivity (after Weyers *et al.*, 1987). Responses are modelled using the Morgan-Mercer-Flodin equation and presented on log-linear axes. Curve A represents a hypothetical primary dose–response curve, in this case fitting the Michaelis–Menten equation, where the interaction coefficient p takes a value of one. Curve B shows the effect of increasing R_{AMP}, a change that would be expected, *inter alia*, to follow from an increase in the number of receptors (other things not being limiting and assuming positive regulation, see section IX). Curve C demonstrates the effect of reducing the interaction coefficient below one. This could result from a number of possible changes in receptor properties, including allosteric binding effects, and has the effect of making the dose–response relationship subsensitive and hence less switch-like. Curve D illustrates the effect of decreasing H_{50}, an alteration that might, *inter alia*, result from an increase in receptor affinity or from second messenger amplification. This type of sensitivity change is often assumed, but is not the only one possible, as can be seen from curves B and C and from those dose–response relationships that could be imagined if combinations of the changes illustrated in curves B-D were to occur. While it is possible to suggest molecular reasons for changes in the shape of D-R curves as above, there can be no certainty to these inferences due to this complexity.

(Sembdner *et al.*, 1980; Hartung & Slovik, 1991). As a complicating issue, some plant hormones act by affecting H^+ transport which may itself then alter the compound's concentration in relevant compartments (Paterson *et al.*, 1988).

Researchers generally use one of two basic methods to obtain D-R curves. Each assumes that the physiological system is initially at a steady state and that conditions other than the hormone concentration [H] are held constant throughout. In both cases, [H] is altered across a concentration range to produce a D-R curve. The methods differ in the measure taken to quantify response.

The initial rate method involves making a step change in [H] and measuring R, the initial rate of change in response. A lag time may need to be accounted for because of the time taken for penetration of hormone to the active site or for the molecular response to be transduced into an observable change

(Weyers *et al.*, 1987). Because of the complexity of cells, tissues and organisms, analysis of initial rates is necessary to ensure that the change in state is not modified by other factors (Cleland, 1972). The results are of value in determining the characteristics of that part of the overall system interacting directly with the hormone. Comparison of such curves allows changes to be distinguished in what Weyers *et al.* (1995) called 'intrinsic sensitivity'.

The steady-state method involves making a step change in [H] and recording the new steady-state value of response once the system has equilibrated. This represents the response to a change in stimulus after all feedback processes have been accommodated by the system and is a measure of the whole system's ability, under the conditions of the assay, to respond to the change in [H] via both direct and indirect effects. Comparison of such curves allows changes to be assessed in what Weyers *et al.* (1995) termed 'equilibrium sensitivity'.

The approach of Weyers *et al.* (1987, 1995) has been applied to a variety of plant hormones acting on a wide range of plant systems. Stomatal sensitivity to abscisic acid (ABA) has been demonstrated to be affected by pH changes (Paterson *et al.*, 1988) and by different watering regimes (Peng & Weyers, 1994). Fitzsimons *et al.* (1988) and Fitzsimons (1989) have characterized the sensitivity of coleoptile H^+ extrusion to natural and synthetic auxins, while Paterson (1989) has investigated IAA effects on stomatal aperture. Sensitivity of *Sisymbrium* germination to nitrate, light and gibberellin was investigated by Derkx & Karssen (1993). Brassinolide-induced seedling elongation and its interaction with auxin has been studied by Sasse (1990). Sensitivity to ethylene has been shown to be important in submergence-induced shoot elongation (Banga *et al.*, 1996; Voesenek *et al.*, 1996). Dwarf mutants of barley have been characterized by means of gibberellin D-R curves (Chandler & Robertson, 1999) and Kappers *et al.* (1998) have used such curves to investigate the sensitivity of leaf senescence to different gibberellins and light quality.

As noted above, the fact that some auxin-coleoptile response curves were bell-shaped was a possible complication for this type of analysis. One possible reason is that these curves were not obtained using initial rates of response (Cleland, 1972; Weyers *et al.*, 1987). In addition, the D-R curves of coleoptile segments treated with IAA are known to depend strongly on the length of treatment (Nissl & Zenk, 1969; Cleland, 1972). They are also affected by the time between excision and auxin treatment and the pH of the medium (MacDowall & Sirois, 1977). Further, responses may be influenced by secondary ethylene production in the long term (Mulkey *et al.*, 1982; Yang & Hoffman, 1984).

Trewavas (1991) and Bradford & Trewavas (1994) postulated that one reason for the wide activity ranges of plant hormones could be variation of sensitivity among populations of cells. They cited several pieces of evidence pointing to this, especially in systems that were heterogeneous in nature. They pointed out the possible effects of nonhomogeneous response on D-R curves and hence on estimates of sensitivity. Bleecker & Kende (2000) noted that receptor isoforms with different affinities for ethylene might act to extend the dynamic range of ethylene action. Nissen (1985) proposed that some elements of the shape of dose–response curves could be explained by a multiphasic D-R relationship. Schistad & Nissen (1984) also put forward a very complex multioptimal relationship in one case. However, neither of these ideas has been widely accepted and A'Brook (1989) strongly criticised the underlying analyses on several statistical grounds, including the fact that too few data points were generally used and that zero error was effectively assumed in response measurements.

IX. Roles of receptors, second messengers and signal amplification in hormone sensitivity changes

Implicit in the notion of chemical control is a chemical interaction at the site of action that is transduced into a response (Eqn 1). This initial interaction is generally held to be mediated by protein receptors (Kende & Gardner, 1976; Stoddart & Venis, 1980; Venis, 1985; but see also Trewavas & Jones, 1981 for discussion of some alternative ideas). Stoddart & Venis (1980) proposed four criteria to establish whether a candidate receptor was involved in hormonal control.

1 The binding of hormone to receptor should be of high affinity, with the saturation range of the receptor being compatible with the concentration range of the hormone known to be active physiologically.

2 Binding specificity for hormone analogues should be in approximate concordance with the relative biological activities of the compounds, remembering that other factors may contribute towards overall activity.

3 Binding should be of finite capacity and should be reversible, although there may be differences in the rates of association and dissociation.

4 Binding should lead to a hormone-specific response.

Approaches to receptor biochemistry have involved various approaches corresponding to criteria 1–3: isolation of receptor activity; analysis of binding data, generally using the Michaelis–Menten equation as a model; investigation of structure-activity relationships; attempts to locate receptors within cells or to subcellular fractions; and studies with artificial membrane systems (Stoddart & Venis, 1980, Venis, 1985). However, the methods required are difficult and the plant material somewhat intractable, and, as Kende (2001) pointed out, these approaches have 'generated far more frustration than results'.

Stoddart & Venis (1980) held that criterion 4 was the hardest to establish without specific receptor mutants. Since that review, the discovery of a number of response mutants for ABA, ethylene and gibberellins has helped to elucidate many aspects of the complex pathways involved (McCourt, 1999). In particular, details of the ethylene response system have been pieced together (Bleecker & Kende, 2000; see below). Further

advances can be expected following the publication of the *Arabidopsis* and *Oryza* genomes, allowing comparison of putative gene products with proteins from other organisms whose roles in transduction pathways are known (Kende, 2001). For example, study of the *Arabidopsis* genome has revealed over 340 genes for serine/threonine kinases (The *Arabadopsis* Genome Initiative, 2000), some of which may act as receptors (Hardie, 1999) and at least eight genes for histidine kinase two-component receptors, five of which are for ethylene (Hua *et al.*, 1998).

Among the 'plethora of surprises' arising from the study of response mutants (Kende, 2001) is the complexity of events that take place. The well-researched ethylene response system in *Arabidopsis* was reviewed in detail by Bleecker & Kende (2000). The sequence of signal transmission so far elucidated involves first, ethylene binding to a two-component receptor, with a copper ion at its active site, that acts in concert with a 'RAF-like' kinase, CTR1, to negatively regulate a membrane protein with a possible metal transport function. Consequent to this is the activation of a family of primary nuclear transcription factors that themselves promote a second transcription factor that activates responses through mechanisms at present unknown.

Especially intriguing features are: the negative regulation, implying that ethylene binding switches a functioning pathway off, so that an increase in the number of ethylene receptors would actually reduce ethylene effects; and apparent redundancy in several of the pathway elements, which may protect against mutation and offer control opportunities via differential expression and/or differences in affinities (Kende, 2001; Bleecker & Kende, 2000). In agreement with the notion of spatial and temporal changes in sensitivity, the ETR family of ethylene receptor proteins has been shown to be expressed differentially in different tomato plant organs and tissues and in specific tissues during their development (Lashbrook *et al.*, 1998; Tieman & Klee, 1999).

The genes for the ethylene receptors may have a cyanobacterial origin, with no homology evident in animal or fungal genomes, while those for downstream components do have similarities to other eukaryote genes. Modules of cellular signal networks from bacteria and animals have been combined and new cascades have evolved in plants. This indicates that plant hormone response systems have a mixed and therefore richer heritage compared to those of animals (The *Arabidopsis* Genome Initiative, 2000).

Changes in sensitivity might occur for many reasons connected with events during and after hormone binding (Fig. 1): they could occur *inter alia* because of changes in receptor numbers; differences in binding efficiencies among receptors; and different linkage to downstream events that determine response (Tieman & Klee, 1999). Bleecker & Kende (2000) pointed out the key role of the CTR1 kinase, which apparently acts to receive all receptor isoform inputs, meaning that the different ETR receptors contribute quantitatively but not qualitatively to the output of the pathway. Hardie (1999) likened such kinases to a central processor unit, accepting hormone and other inputs, and processing these into appropriate metabolic outputs. It seems likely that these enzymes could play an important role in adjusting plant hormone sensitivity, especially over shorter timescales.

A variety of novel techniques has led to progress in determining the role of second messenger systems in plant hormone responses (Webb *et al.*, 1996). The involvement of changes in cytosolic Ca^{2+} (Ca^{2+}_{cyt}) in the ABA response of stomata is particularly well characterized and Ca^{2+}_{cyt} has been shown to inactivate inward rectifying K^+ channels, an event important for stomatal closure (McAinsh *et al.*, 2000). Similar events may occur in other ABA-induced responses such as desiccation tolerance and inhibition of germination (Leung & Giraudat, 1998). In many ways the involvement of Ca^{2+}_{cyt} and associated mobilizing pathways parallels that of signal transduction in animal cells (McAinsh *et al.*, 1991, 2000). Intriguingly, however, a rise in $[Ca^{2+}_{cyt}]$ occurs whether the stimulus opens or closes the stomata. The appropriate direction of response might be encoded by an interaction with pH, by a spatial and temporal $[Ca^{2+}_{cyt}]$ signature, or other second messengers may be involved (Webb *et al.*, 1996; McAinsh *et al.*, 2000). Studies on ABA-insensitive *Arabidopsis* mutants may throw further light on the precise signal transduction mechanism.

Clearly there are multiple opportunities for modification of stomatal sensitivity to ABA within these pathways and the whole story may include additional factors such as 'physiological address'. This was defined as the range of signalling elements present in the cell at the time of exposure to stimulus, and it is thought to affect ABA-induced $[Ca^{2+}_{cyt}]$ changes and transduction pathways (Allan *et al.*, 1994; McAinsh *et al.*, 2000). The situation is complicated by the existence of several transduction pathways: in fact, Jenkins (1999) favoured the term signal transduction 'network'. He reviewed negative regulation, synergism and cross-talk within the networks present in plant cells as part of their responses to environmental stimuli.

Some useful insights about the roles of receptors and second messenger systems in sensitivity changes can be made via modelling. Given reasonable assumptions about binding and postbinding events, Eqn 2 or a similar model can provide useful conclusions about the effects, for example, of:

1 Changing the number of receptors. Other things being equal and there being no other limitations on the ability to respond, an increase in the number of receptors would cause a change in R_{AMP} in Eqn 2, and an increase in the magnitude of the rate of response (Fig. 3);

2 The affinity of the receptor for the hormone. This could occur because of effector binding or because of a change in the expression of different classes of receptor, or a change in their relative activity. A change in affinity would cause a shift in the D-R curve along the [H] axis (Fig. 3);

3 The existence of second messenger systems. Strickland & Loeb (1981) noted that secondary binding events would be

likely to shift the D-R range away from that of the receptor binding curve, offering an explanation of discrepancies under category 1 of Stoddart & Venis (1980). Given that secondary messengers generally act so as to amplify the hormone signal, the predicted effect is to shift the D-R curve lower on the concentration range than the corresponding receptor binding curve.

This modelling approach has been attempted by several workers, including Firn (1986), Weyers *et al.* (1987, 1995) and Trewavas (1991). However, while changes to D-R curves and hence sensitivity can be predicted from receptor properties and postbinding events, the reverse is not true. There are many possible reasons why a curve might change, so alterations in properties of receptors or other response components cannot be directly inferred from differences in D-R curves, although these might give useful clues to direct research (Weyers *et al.*, 1995). When binding data for hormone receptors become available, then comparison of binding kinetics with higher-level dose–response curves could yield useful mechanistic clues, for example, concerning the functional significance of amplification systems in the D-R chain (Strickland & Loeb, 1981; Loeb & Strickland, 1987; Paterson, 1989).

No doubt the future will bring many revelations concerning receptors, subcellular signals and amplification systems. Exciting though this progress is, evidence at the molecular level cannot reveal fully how plant hormones act at the organ or tissue level. Mechanisms of action at these scales can be considered to some extent independently from mechanism of action at the molecular level. For instance, knowledge of the existence of hormone synthesis pathways in one tissue and of a complicated response catena in another means relatively little if we cannot provide evidence that the two are linked in a physiologically meaningful way. There needs to be reconciliation of the properties of receptors and signalling components with the response *in vivo*, for example with respect to speed of response and the kinetics of receptor binding and of release from binding (Bleecker & Kende, 2000).

It should also be borne in mind that the summation or interaction of responses in different cells determines the overall tissue response. Differences in cell or tissue sensitivity over less than 0.1 mm can greatly influence the final outcome, as in the guard cell response to ABA (MacRobbie, 1991) and the *Avena* coleoptile response to IAA (MacDonald & Hart, 1987). Hence, differential responses can give rise to a large change in response for a small change in concentration or sensitivity due, for example, to levering or steering effects. A biophysical understanding of the response may be of great importance in understanding its evolutionary selection value.

X. Relative control as a pivotal concept

A powerful idea that has been put forward in plant physiology is that hormones are in some way the limiting factors in physiological processes. If activity is stimulated when exogenous compounds are applied, then this appears a superficially logical deduction. However, much more needs to be shown before this conclusion can be validated. For instance, the internal concentrations resulting from external application must be shown to be realistic and changes between low (limiting) to high (releasing from limitation) concentrations must be observed during the natural phenomenon. The greatest objection to this concept comes from the fact that most physiological phenomena are influenced by several factors in addition to hormones.

McIntyre (2001) has taken the view that these other factors, such as nutrients, are in fact the important limiting substances, whereas hormones are not. However, as Trewavas (1986a, 1986b) and Zucconi (1988) have pointed out, the idea of a single limiting factor, whatever its nature, is unlikely to apply to complex interconnected systems like those resulting in plant responses. Physiological phenomena certainly involve many inputs that can influence the response. These can be environmental stimuli as well as internal developmental signals. Thus, stomata respond to light, CO_2 and ABA, and many other signals (Willmer & Fricker, 1996) while the response to ABA can be moderated by nitrate and phosphate nutrition (Radin & Ackerson, 1981; Radin, 1984), enriched CO_2 (Radin *et al.*, 1988), high and low temperatures (Trewavas, 1987; Rodriguez & Davies, 1982) or past stress episodes (Ackerson, 1980, Peng & Weyers, 1994). Apical dominance, seed germination and growth rate are further examples of phenomena also influenced by many different factors and by changes in sensitivity to hormones (Trewavas, 1986a).

In two reviews, Trewavas (1986a, 1986b) highlighted two of the general features of complex network systems. First, as noted above, he recognized there is rarely a single limiting process and hence no single control point. As Trewavas (1986b) put it: 'classical hormone theory confuses playing "a" role with playing "the" role'. Second, he emphasized that responses to perturbing one part of the pathway are not easily predicted and may in fact be counterintuitive. On this basis, Trewavas claimed that plant hormones are unlikely to be good controllers for complex pathways, and suggested network theory as a route to understand chemical control in plants, drawing a comparison between work on metabolic systems, as modelled by Kacser & Burns (1979), and hormone response systems.

Of course, this analogy is not perfect. In the plant hormone field it is not always 'metabolic control which is being investigated' (Trewavas, 1986b), as several hormones seem to act on membrane transport systems that do not (directly at least) involve metabolism or control over gene expression. The obvious examples are the effects of IAA on proton extrusion in the acid growth mechanism and of ABA on stomatal ionic relations. More fundamentally, however, hormones are not components of a pathway, linking pools of metabolites in the same way that enzymes do. The analyses of workers like Kacser & Burns (1979) were intended to cover product formation in complex metabolic pathways, and thereby identifying which one of

several enzymes in the pathway might be controlling output (the thesis being that control is usually shared). The metabolic sequence leading to a detectable phytohormone response might be regarded as a network, but the possibility that hormonal control systems might have evolved precisely to counteract the 'shared control' and 'resilience to perturbation' aspects of such complex systems (Trewavas, 1986b) seems to have been ignored. The third flaw in this line of thought is that if classical hormonal control is not a viable proposition for complex pathways, then why has it evolved so successfully in animal systems? Although these systems are different qualitatively, as discussed above, they surely cannot be regarded as either quantitatively more or less complex in detail from plant systems. Logically, therefore, complexity alone cannot be adduced as an argument against hormonal control.

The better analogy might be with control system theory rather than with metabolic network behaviour, although in effect both can be approached by investigating the control sensitivity, as suggested by Trewavas (1986b). Trewavas (1991) proposed the use of sensitivity analysis, as developed for engineering problems. These techniques have been adopted for biochemical pathways (Kacser & Burns, 1979) and ecological systems (Patten, 1976), as well as for some situations in plant physiology such as control of photosynthesis (Jones, 1985) and transpiration (Weyers & Lawson, 1997). The basic notion behind sensitivity analysis is to manipulate factors up or down from a baseline value (presumably one that is physiologically relevant) and examine the effect on the output, reaction or response of interest. More formally, the tangent of the D-R curve is estimated at the baseline value. By doing this for all factors, but keeping the others at their original baseline values, the relative effect of each stimulus can be assessed.

Trewavas' control strength concept (Trewavas, 1991) involved normalizing both the response and the concentration (factor value) such that the maximum response and factor value were defined as 1. The slope of this relationship at any given factor value (such as that predicted *in vivo*) gives the fractional control exerted at that value. Trewavas reanalysed published data for gibberellin effects on leaf length, finding that the control strength was less than 0.05; he concluded that for plants growing at near maximal rates, changes in gibberellin concentration would not have great effects on growth rate.

Paterson (1989) and Weyers *et al.* (1995) developed an alternative, but essentially analogous, system of analysis. They defined the term 'control coefficient' as the slope (dR/dLog[H]) of the D-R curves described by Eqn 1 in semilogarithmic plots. Plotting the control coefficient against [H] results in a bell-shaped curve with a maximum at $[H] = [H]_{50}$. The potential influence of the compound is greatest at this point and declines at concentrations either higher or lower. Paterson *et al.* (2001) extended this analysis to include other influences as well as hormones (globally termed 'factors' and denoted by the symbol F). They noted that that the units of the control coefficient are those of the response rate and are thus independent of the units of [F] and that the control coefficient can be defined generally as the slope of the D-R curve, whatever the particular function describing that curve.

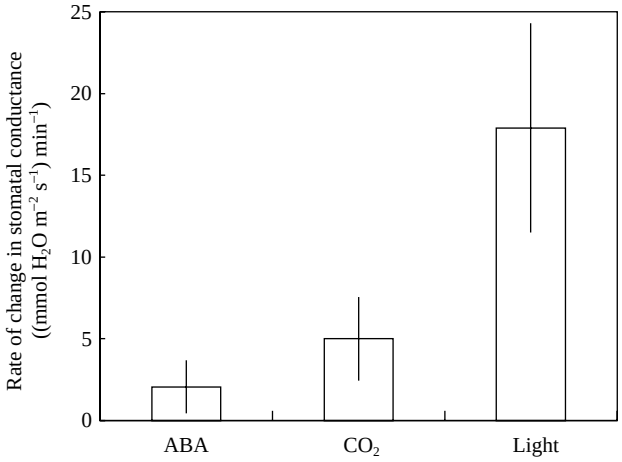


Fig. 4 Relative control coefficients for ABA CO_2 and PPFD. The coefficients were determined at specified baseline conditions of: ABA, 4×10^{-4} mol m^{-3}, corresponding to those in the xylem sap of 'unstressed' plants; CO_2, 355 ppm, presumably giving a Ci value close to 230 ppm; and PPFD, 318 mol photon m^{-2} s^{-1}, equivalent to *c.* 15% of full sunlight. Each coefficient was estimated as the slope of the partial log-lin dose–response curve at the baseline value of the relevant variable (Paterson *et al.*, 2001). Bars represent 95% confidence limits.

If a factor is present at a level coinciding with a steeper part of the D-R curve, that is, where the control coefficient is higher, the potential to effect control by changes in level is greater than where the baseline value coincides with a lower control coefficient (Weyers *et al.*, 1995). By assembling D-R data for a variety of factors under defined and relevant conditions, the relative control potential of these factors with respect to the response under study can be assessed. The concept of relative control is connected with the narrower problem of sensitivity, because the degree of control a given factor exerts on a process depends on the sensitivity of the process to the factor.

Paterson *et al.* (2001) used the stomatal response to investigate this problem. They chose three differing stimuli – ABA, CO_2 and light – and defined a 'baseline' set of conditions for all three that were related to natural levels of interest. Step changes were made to [ABA], to CO_2 and to light under this otherwise defined and constant set of conditions, and the change in rate of stomatal response determined to give an estimate of the slope of the D-R curves. The findings were perhaps surprising: in unstressed *Phaseolus* leaves under relatively shaded conditions, ABA was potentially the least effective controller (Fig. 4). At least superficially, this finding does not fit with the notion of ABA acting as a classic hormone providing a signal from the roots to the shoots to indicate drying soil conditions (Zhang *et al.*, 1987). On the other hand, it is consistent with the findings of Munns & King (1988), who demonstrated that ABA is not

the only stomatal inhibitor in the transpiration stream of water-deficit stressed wheat plants. However, the likelihood of finding a higher ABA control coefficient under different conditions cannot be excluded and may even be considered likely (Paterson *et al.*, 2001). There is strong evidence that exposure to prior water deficit stress alters the sensitivity of stomata to ABA (Peng & Weyers, 1994). Also, stomatal sensitivity to ABA can be manipulated by pH changes (Paterson *et al.*, 1988), and pH changes in xylem sap are associated both with water stress and ABA-mediated stomatal responses (Hartung *et al.*, 1988; Wilkinson *et al.*, 1998).

The existence of other influences on a response is expected under the relative control philosophy, where hormones are seen as acting in a milieu of other influences. The approach taken by Paterson *et al.* (2001) demonstrates that the control coefficients can be used to rank factors impinging on physiological processes in order of importance, thus highlighting those which are more or less important in a given system, under particular conditions. This approach can also be adapted for other systems, choosing baseline conditions, response indicators and plant material relevant to any particular hypothesis.

XI. Diversity of physiological roles for chemical influences in plants

Trewavas' (1981) criticism of simplistic hypotheses for hormone action may have seemed unfair to some, because they were already aware of the inherent complexities involved in the field. Careful reading of pre1981 papers often reveals discussion points and caveats that demonstrate this deeper understanding. Nevertheless, some publications before and since this time seem to proceed with experimentation without any reference to a conceptual framework, or else make sweeping assumptions about the involvement of hormones that are not necessarily supported by evidence. By emphasizing some problematic observations that were otherwise ignored, Trewavas highlighted the lack of an underlying philosophy that could accommodate observations not satisfactorily explained by the classic hormone theory. What was required thereafter was one of two things: either a completely new hypothesis, or one that modified the core hormone hypothesis to include the complexity.

Trewavas himself attempted to fashion new notions of phytohormone action to replace the 'synthesis-transport-action' model (see section II). So far, none appears to have been widely adopted. Since his intervention, however, there has been a concerted attempt to include the concept of mutable sensitivity alongside the idea of chemical control. Essentially, combined control as defined in section V is accepted to exist in one form or another in all cases – but this is perhaps not enough to reconcile the differing viewpoints. It remains difficult to see an overarching or all-encompassing view that satisfactorily explains what advantage hormones provide to plants. Perhaps such attempts to force plant hormone physiology into a straitjacket are futile. It is conceivable that natural selection has resulted in a multiplicity of roles, even for the same compound as it acts in different phenomena (Leopold & Noodén, 1984). Natural selection favours those genomes whose phenotypes respond in such a way that they will be the most likely to survive. In evolutionary terms, it matters little how this is achieved physiologically, so long as the mechanism satisfies other selection pressures such as the efficient use of resources, and can be fashioned by mutation of the genetic material to hand (Ketterson & Nolan, 1999; Meyerowitz, 1999).

Thus, if a 'classic' hormonal role is beneficial, then it will survive; if a different role fits the need, then this will persist. If physical factors are sensed directly, then these may be involved in control alongside the hormonal input. Furthermore, it is also possible, even likely, that natural selection has acted to overlay response modes during separate evolutionary events, with the same discriminatory forces working to ensure that they function in an integrated and effective, albeit complex, manner. Scientists like to think of things in simple terms, mainly because uncomplicated hypotheses are aesthetically satisfying, attractive to communicate and simple to test experimentally. Scientific history includes many examples of simple notions that have elucidated a field, and thereby enabled progress, but that have been corrected or modified at a later date when a more complex picture emerges as evidence accumulates.

Examination of the literature indicates that hormonal involvement in physiological processes can appear in several distinct manifestations. Below are outlined 12 distinct roles, and more may be possible. These will be discussed briefly, with emphasis on the defining characteristic that marks each out and the crucial element(s) of the frameworks noted in section V that would be most important in allocating a phenomenon to the category (this is not to diminish the importance of examining the other criteria, too). At least one example of each type of role will be given, but the analysis will be brief.

1 Distance signal. This is the classical 'synthesis-transport-action' role, where a physiological message is sent between two locations, eventually taking the form of a change in the concentration of chemical at the site of action. The hormone moves between a site of synthesis and a site of response, co-ordinating the activities of different tissues or organs. If this role is hypothesized, there is a concomitant duty to explain what advantage may accrue from the separation of the sites of perception and response – why not simply perceive the stimulus at the site of action? As in other cases, such an analysis should take into account the possibility that a complex solution may have prevailed because of the nature of pre-existing structures and functions. Transport of hormone would need to be demonstrated for this role. Further, the underlying assumption of concentration-dependence at the site of response (chemical control) needs to be quantitatively demonstrated (see section VII). Sensitivity might be important in the response either because specific target cells respond or because differential acts

to amplify the response. Examples of hormone action apparently involving translocation were cited by Cleland (1983), (see section II), while Jackson (1993) reviewed several phenomena potentially involving a root-shoot hormone-like signal, including leaf senescence and shoot responses to flooding and drought.

2 Enabling substance. This role, as a stationary signal transmitter (section III), follows Trewavas' (1981, 1982) 'extreme' hypothesis, where sensitivity dominates and competence to respond dictates the response. Changes in hormone level are not an important feature – instead, control resides with the tissue in terms of changes in sensitivity (Trewavas, 1986a) and the hormone titre in an organ has a permissive role in the sense used by Barlow (1987). Trewavas (1981) proposed that this mechanism was important for plants because of their phenotypic plasticity. Certain cells are envisaged to be sensitized to respond either during a developmental sequence or following external events. Trewavas (1986a) respectively, termed these modes 'ontogenetic sensitivity' and 'metabolic sensitivity'. He saw the primary role of the latter in resource allocation under poor growth conditions (distinguished here in the category 'Resource Allocator' in category 10 below). For a role involving ontogenetic sensitivity, it should be possible to demonstrate that the hypothetical changes in sensitivity can quantitatively explain the difference in response. There is an onus to explain why the response has not evolved such that the responding cells react directly to the stimulus rather than via the intermediary of the hormone, with the same caveat as noted in category 1 above, and to explain the selective advantage this sort of response offers. Several examples of changes in ontogenetic sensitivity were provided by Trewavas (1982), including: changes in sensitivity of coleoptile cells to auxin and cytokinins correlated with cell age and position (Wright, 1966); variations in ethylene sensitivity during tomato fruit ripening (McGlasson & Pratt, 1964); and alterations in sensitivity of barley aleurone cells to gibberellins (Trewavas, 1982).

3 Stimulus integrator. Here, the notion is that the hormone 'pool' acts as the shared means of triggering a single reaction where multifold signal inputs may operate. Several stimuli are envisaged as capable of modifying hormone production (and/or sensitivity) yet only one response results. This would allow stimuli to act in concert to force a response, where none might do so individually. The experimental requirement here would be for stepped introduction of different stimuli to lead to a response either due to changes in the concentration of hormone or sensitivity to it. This mode of action might have advantages for phenomena like the onset of dormancy or release from it, where an individual stimulus event (e.g. temporary change in temperature) may not be 'reliable' as a predictor of season, but where the likelihood of predicting the season's onset might be improved by taking several such indicators into account or summing events for an individual type of stimulus. Thus, many factors influence seed dormancy and may combine with sensitivity changes to exert control in this way via gibberellins or abscisic acid, or a balance of these compounds (Derkx & Karssen, 1993; Karssen & Lącka, 1986).

4 Response co-ordinator. The focus here is on the plant obtaining advantage from setting in train many physiological responses to a single developmental or environmental event, with a change in hormone concentration being the common signal in all cases. An environmental change might require co-ordination of several responses of differing types, some perhaps being short-term, others long-term (see also category 5 below). Or, there may be a need to set in train one type of response in one tissue, but another in a different one. This form of control would require demonstration of a stimulus affecting the hormone concentration. It could include differential sensitivity or sensitivity changes, but if these predominate, the example might be better considered under the 'enabling substance' category (category 2 above). There would not need to be translocation of the compound, but there would need to be correlation of multiple physiological responses with changes in hormone concentration. These possibilities could be assessed by relating changes in endogenous hormone concentration to the D-R curves for the different phenomena. An example of this form of control might occur in water deficit stress. If a drop in turgor below a certain threshold leads to a change in ABA concentration, this could trigger a range of physiological responses from stomatal closure to synthesis of osmoticum (see Trewavas & Jones, 1991).

5 Temporal message. Plants, being generally slower growing and responding than animals, may have a corresponding need to provide long-lived signals in time. In this case, the hormone is seen as an enduring message, possibly remaining active long after the immediate and possibly temporary stimulus has disappeared. This role may be combined with that of response co-ordinator (category 4 above), if several responses are involved that differ in induction time. This role implies a change in hormone concentration that would persist after the stimulus had occurred. Translocation might be required to allow for responses in different parts of the plant. An example of this category might be wound or defence hormones, as involved in hypersensitivity and acquired resistance (Keen, 1999). Thus, a single herbivore attack might be short-lived, but the plant could benefit from 'priming' against future attacks through an induced systemic defence system. This might co-ordinate events like localized cell death or chemical changes that affect herbivory or pathogenicity, such as production of distasteful/poisonous compounds or inhibitors (Hanson & Trewavas, 1982). Pheromone-like activity may occur, so crossover with role 11 (category 11 below) is possible. Possible benefits of induced resistance to herbivory were discussed by Karban *et al.* (1997) and Baldwin (1998). Theoretical studies are in accordance with Trewavas (1986a) in the view that chemical defence is an efficient strategy when resource availability is low (Yamamura & Tsuji, 1995). The polypeptide hormone systemin is an example of a hormone acting in this manner (Pearce *et al.*, 1991) as are salicylic acid and the jasmonates (Pierpoint, 1994;

Kombrink & Somssich, 1995; Creelman & Mullet, 1997). These hormones accumulate in wounded plants and *inter alia* induce the synthesis of chemicals and protease inhibitors that protect against herbivory. There may be complex interactions between several wound response systems that involve hormones (Creelman & Mullet, 1997; O'Donnell *et al.*, 2001).

6 Spatio-temporal synchroniser. Here, the hormone acts over a dispersed area and long time to ensure uniform synchronized reaction to a stimulus where spatial and temporal heterogeneity in response might otherwise exist. The hormone is seen as a 'smoothing agent', ensuring that all cells get the same message, or arrive at the same developmental point at the same time, regardless of their individual microenvironment or developmental stage. Trewavas (1983b) likened this form of control to that of a flywheel on a motor, counteracting the tendency for erratic response to stimuli with a high degree of inherent variability. As Bradford & Trewavas (1994) point out, this can involve sensitivity changes as well as changes in hormone level. In addition to the normal criteria there would presumably need to be a statistically based demonstration that the action of the hormone resulted in lowered dispersion of responses. As discussed by Bradford & Trewavas (1994), an example might be in the coordinated ripening of a fruit resulting from the stimulation of the climacteric by ethylene production (McGlasson & Pratt, 1964). Here, selective advantage can be seen because a uniformly ripe fruit would be more likely to be eaten by an animal seed-dispersal vector (Bleecker & Kende, 2000).

7 Overriding controller. The notion in this case is that control over a response might normally reside in a complicated array of stimuli, but there is a need for an 'executive' control message that will override all others during a physiological emergency. For this role, the strength of a given signal in relation to others (relative control potential) would need to be assessed and this may itself alter (via changes in sensitivity) in a form of combined control. This type of control could occur in the control of stomatal aperture during water stress. The onset of a sudden and severe water stress results in loss of cell turgor due to the drop in water potential. Stomata react in a complex way when they lose turgor, and each phase provides a potential hazard to plants. In the initial stage, guard cell turgor loss may result in stomatal opening because of the mechanical advantage of the functional subsidiary cells (Weyers & Meidner, 1990) – obviously contrary to long-term needs. In the later stages of guard cell turgor loss, the stomata may start to close, but, although this reduces water loss, it simultaneously presents a problem because stomatal closure in the presence of continuing photosynthesis would result in lower C_i, providing a contradictory signal forcing stomatal opening. The plant could thus benefit if ABA, as the messenger of a physiological emergency, could override all the other signals (Weyers & Peng, 1995). At high concentrations, the compound is known to be able to close stomata even in CO_2-free air (Weyers & Hillman, 1979b). However, this role remains hypothetical, because for unstressed plants under low light conditions ABA does not have a high relative control potential (Paterson *et al.*, 2001). This could change in high light or during a stress (see references cited in section VIII). This role could also be considered an extreme manifestation of the preceding role 6 (category 6 above) in the context of heterogeneity in stomatal aperture (Weyers & Peng, 1995; Weyers & Lawson, 1997).

8 Homeostatic regulator. Here, the hormone acts as a regulatory signal within a feedback system to maintain some physiological process according to an independently determined reference value. This is regulation in the sense meant by Cram (1976) as discussed in section III. For this role, there would need to be evidence of a 'switch-like' change in hormone synthesis as conditions changed and one would expect to see 'hunting' behaviour (oscillations in response) typical of feedback regulation, correlated with changes in hormone concentration and/or movement. The normal criteria for demonstrating chemical or combined control would be applicable in this case. ABA might act in this way to maintain positive leaf turgor. A number of different pieces of evidence indicate that the signal for ABA production is loss of turgor rather than the crossing of some water potential or solute potential threshold (Davies *et al.*, 1981; Davies & Jones, 1991). If the ABA production so induced caused stomatal closure, this would increase plant turgor, helping to maintain homeostasis in that parameter. It should be noted, however, that loss of leaf turgor is a rare physiological event, possibly because of predictive spatial signalling from the roots (Zhang *et al.*, 1987).

9 Environmental sensor. In this category, the hormone acts to help the plant sense its surroundings. The hormone diffuses away from the plant at a rate that provides information about the prevailing environment when the outside concentration is sensed by the responding tissues. Requirements to demonstrate this role include evidence of environment-related changes in rates of diffusion and of a sensing system capable of responding to the external concentration. This mechanism has been proposed to act during emergence of germinating dicot seedlings, where the gaseous hormone ethylene is thought to control growth patterns (Smalle & Van der Straeten, 1997). The gas is produced by the plant continuously and diffuses into the soil air spaces. Its concentration provides an indication whether the plant remains constrained by soil (high concentration) or has broken into free air (low concentration). Epicotyl thickness and growth rate, as well as plumular hook uncurling, all of which are affected by ethylene concentration, are thereby coordinated with emergence from the soil, this sensing system being allied to another that responds to the quality of light as an indicator of emergence, via phytochrome. There may also be communication between ethylene and IAA response systems (Smalle & Van der Straeten, 1997). Another example in this category would be seed coat inhibitors, whose diffusion away from the seed, being enhanced in periods of rainfall, might provide an indication of the water status of the soil and indicate a beneficial time for germination (Mayer & Poljakoff-Mayber, 1982).

10 Resource allocator. Trewavas (1986a) presented a strong case that plant hormones only become important controllers under poor growth conditions (i.e. imbalances in light, nutrition, water availability and O_2 supply), where developmental plasticity could provide benefits through altered growth and form that optimizes the response to the conditions. Trewavas proposed that these responses were brought about through acquired sensitivity to the relevant hormone due to the change in conditions ('metabolic sensitivity'). For this role, a change in sensitivity is required following an imposed stress, possibly combined with changes in hormone concentration. The response to the stress should involve a growth or developmental response that fits quantitatively with control via sensitivity or combined control caused by these factors. The response should provide some alleviation of the stress. An example within this category is the involvement of ethylene in plant responses to waterlogging and submergence (Jackson, 1985; Voesenek *et al.*, 1996), as in the elongation of *Rumex palustris* petioles following flooding (i.e. low O_2). This response increases the probability of survival by restoring leaf air contact and involves both changes in ethylene concentration and differential sensitivity to ethylene between young and old leaves (Banga *et al.*, 1996).

11 Inter-plant signaller. This category encompasses both allelochemicals and pheromones as molecules that signal between individual plants (of different or similar species, respectively). There is presumably a strong local effect that diminishes greatly with distance, so to show this type of action, evidence would be required that the compound could elicit a response at the concentrations normally found in the environment (Newman, 1983). Elimination of other possible controlling influences can present logical and experimental difficulties, especially for allelochemicals (Williamson, 1990). Some benefit to the signal-initiating individual or to the gene pool would need to demonstrable as a result of the behaviour pattern. Allelopathy frequently involves inhibition of the growth or metabolism of another plant or germination of its pollen, pores or seeds (Rizvi *et al.*, 1992) and it seems self-evident that it would reduce competition. There is a wide range of allelochemical compounds, including many phenolics (Rice, 1984). In higher plants, pheromone action may involve a warning aspect, for example of insect attack, and they can induce responses that may reduce herbivory. A pheromone role has been has been suggested for methyl jasmonate in *Artemesia* and methyl salicylate in *Nicotiana* (Farmer & Ryan, 1990; Shulalev *et al.*, 1997).

12 Biotime adjuster. This role was suggested by Bradford & Trewavas (1994). The idea is that hormones allow plants to speed up or slow down biological time ('biotime') by altering the rates at which physiological processes occur. The notion is analogous to the inverse relationship between temperature and development, where the developmental biotime scale increases or decreases according to the prevailing temperature. Bradford and Trewavas propose that hormone concentration can affect biotime in a similar way. An example quoted by Bradford & Trewavas (1994) in support of this idea is the effect of gibberellin on seed germination in tomato seed (Ni & Bradford, 1993), where an inverse relationship between log [H] and time for germination can be demonstrated.

Is it possible that plant hormones may act in any one of these roles, or a combination, according to the physiological phenomenon being considered and even the stimulus involved? Could this diversity and complexity explain the difficulty botanists have experienced in unravelling the role of plant hormones? Each of the above roles is capable of investigation via experimentation within a framework that appropriately modifies the criteria outlined in sections IV and V. Some roles involve transport, while others do not; some place a premium on alterations in sensitivity, others on changes in hormone concentration. Many can be interpreted as involving combined control and this may indeed turn out to be the norm. The roles should not to be seen as exclusive – some may overlap, as indicated. There is no reason why a plant may not derive benefit from hormone action under several of these categories (e.g. the response of stomata to ABA during water deficit stress fits several categories, as has been noted above). Multiple roles may well be appropriate by providing selective benefit through more than one mechanism. However, a clear notion of what is hypothetically being controlled, and why, is required to aid in design of appropriate experiments and interpretation of data. Thinking in terms of the above potential mechanisms of control (and others, where relevant) might release plant physiologists from the constraints of the classic hormone theory, and might prevent futile debate. Researchers would need to specify in very clear terms which role is postulated, what additional criteria for chemical control might operate in that system and how the particular piece of evidence being reported fits into the framework being applied.

XII. Concluding remarks

An underlying theme of this review has been that plant hormone research has failed to advance as quickly as it might have because difficulties in identifying and following theoretical frameworks, coupled with a general lack of funding to support research, has led to patchy work on disparate experimental systems. Furthermore, research has failed to address critical issues at a quantitative level. There has only been limited exploration of the logical possibilities for modes of actions outside the classic hormone model. Despite sustained criticism of that notion, no philosophically satisfying alternative thesis has emerged, probably because no one answer will suit all cases. Accordingly, this review has presented an alternative and novel view of the role of plant hormones in physiological phenomena. It is, simply, that no overarching explanation is possible, nor should be sought. Instead, it is postulated that there are many different roles to suit different demands and different phenomena. Even the potentially unifying concept

of chemical control may not be universally applicable because of the likelihood of control by sensitivity or combined control.

The evolution of plant hormone responses is a fundamental question that underpins all the topics addressed in this review. It is assumed that the plant will have evolved to utilize hormones in whatever capacity gives selective advantage. The emerging evidence from detailed molecular studies indicates that plants share many aspects of their subcellular control pathways with animals and microorganisms. It is therefore at the supracellular level that differences in the roles of hormones will emerge, consistent with the special features of multicellular organization in animals and plants. Knowledge of the genetics of hormone systems, courtesy of *Arabidopsis* and other organisms whose DNA have been sequenced, will allow these events to be deciphered from a phylogenetic standpoint (Meyerowitz, 1999). Plant scientists therefore need to focus on the 'whole plant' aspects of hormonal control which although not readily understood from studies at the molecular level, may be elucidated by the application of molecular technology. A key issue must be to establish a credible selective advantage for the role of the hormone in the physiological process under study. Following this, appropriate criteria for testing the hypothetical role should be selected and tested. Use of such frameworks in plant physiology allows an individual, research group or scientific community to follow a planned series of experiments. It is therefore important that any report dealing with a subset of evidence should place this in the holistic context, so that its full relevance can be seen, and areas where the evidence remains deficient can be identified.

A sound understanding of the basic physiology and ecology is required before designing experiments or making conclusions: there should be a detailed and quantitative description of all aspects of the phenomenon in question, including perception of the stimulus and nature of the response, as suggested by Firn & Digby (1980) for tropic responses. Next, the analysis should be quantitative and rooted in both molecular biology and ecology, as emphasized by Jackson (1987) and Williamson (1990). Appropriate and quantitatively rigorous methods of measuring hormone concentrations must be employed, as emphasized by Reeve & Crozier (1980) and others, with measurements being made as close as possible to the proposed site of action. These measurements should be quantitatively related to D-R curves as a means of determining the respective roles of hormone concentrations and sensitivity. The assessment of sensitivity through D-R curves should involve appropriate models and valid statistical analysis (Weyers *et al.*, 1987; A'Brook, 1989).

There is need for greater awareness of the contrived nature of experiments and the possible effects of this on results and conclusions. While response systems are expected to be genetically 'hard wired' into each individual organism, plants have great capacity to develop according to their growth conditions. Because biologists are conditioned to think in terms of the canalized development of animals, the special nature of plant systems (section II), and its possible effects on results, may easily be overlooked. The glasshouse or growth cabinet plant, growing in a pot of sterile, well-fertilized soil, in artificial light and controlled temperature and in the absence of wind, pathogens and predators, is not the same as the one growing in the wild, and it almost certainly differs quantitatively and perhaps qualitatively in its responses (Trewavas, 1986a). There is ample evidence, for example, that the responses of plants to ABA are affected by their past experiences (Peng & Weyers, 1994), though this is probably not brought about by changes in the concentrations of the hormone itself (Borel *et al.*, 2001).

There is also the possibility that the experimental isolation of the responding system, although desirable in some respects (see section V), may cause abnormal responses. Wound effects may occur, as notably in the heightened sensitivity to IAA that results from excising coleoptile segments from the intact organ (Trewavas, 1982; Hanson & Trewavas, 1982). Equally, inappropriate experimental conditions can prevent a response. For example, Lancaster *et al.* (1977) reported that stomata of *Lupinus luteus* were unresponsive to ABA and this was taken as evidence against a universal role by Trewavas (1981). In fact, the experiments had been carried using a KCl concentration of 300 mol m^{-3}, far too high for responses to occur even in stomata of species otherwise known to be responsive (Weyers & Hillman, 1979b). Another important issue concerns the manipulation of experimental conditions to emphasize hormonal influences. If relative control potentials for hormones can alter according to past or present conditions (see section X), then a logical consequence is that it must be possible to manipulate conditions to emphasize the hormonal component of a control system relative to other influences. Researchers using isolated experimental systems in the sense of Jacobs (1959) may have unwittingly emphasized the hormone influence or even created completely artificial responses (Zucconi, 1988; Trewavas, 1991).

When analysing the hormone literature, it is sometimes difficult to distinguish between good technique and poor; reliable results and unreliable. Certainly, most researchers are aware that results and conclusions in their area can depend on precise detail of technique. Care needs to be taken to ensure that different research groups follow the same procedures or that different results obtained with different species or methods are investigated thoroughly. In times of limited resources, there is a need for much greater cooperation, multidisciplinary and international if need be, so that effort and resource is pooled towards a common goal. Experts in each field need to analyse past data within the frameworks suggested, perhaps as formal working groups. These could determine what experiments might be valuable to complete the framework of evidence. There needs to be healthy scrutiny of 'outlying' results in the literature, perhaps with a commitment to repeat experiments where the conclusions are important. Finally, although an agreed experimental system for each case would be valuable, possible differences among species should be explored as these

might provide valuable information about the phylogenetic development of hormonal roles.

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References

A'Brook R. 1989. *Some practical aspects of the application of concentration-response models*. PhD thesis. Dundee, UK: University of Dundee.

Ackerson RC. 1980. Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. *Plant Physiology* **65**: 455–459.

Aguinaldo AM, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* **387**: 489–493.

Aldington S, Fry SC. 1993. The oligosaccarins. *Advances in Botanical Research* **19**: 1–101.

Allan AC, Fricker MD, Ward JL, Beale MH, Trewavas AJ. 1994. Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. *Plant Cell* **6**: 1319–1328.

Allan AC, Trewavas AJ. 1994. Abscisic acid and gibberellin perception: inside or out? *Plant Physiology* **104**: 1107–1108.

Audus LJ. 1969. Geotropism. In: Wilkins MB, ed. *Physiology of plant growth and development*. London, UK: McGraw-Hill, 204–242.

Audus LJ. 1972. *Plant growth substances, vol. 1. Chemistry and physiology, 3rd edn*. Valletta, Malta: St. Pauls Press.

Baldwin IT. 1998. Jasmonate-induced responses are costly but benefit plants under attaqck in native populations. *Proceedings of the National Academy of Sciences, USA* **95**: 8113–8118.

Banga M, Blom CWPM, Voeseneck LACJ. 1996. Sensitivity to ethylene: the key factor in submergence-induced shoot elongation of *Rumex*. *Plant, Cell & Environment* **19**: 1423–1430.

Barlow PW. 1987. Requirements for hormone involvement in development at different levels of organization. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 39–51.

Bayliss WM, Starling EH. 1902. On the causation of the so-called periferal reflex secretion of the pancreas. Preliminary communication. *Proceedings of the Royal Society* **69**: 353–353.

Bearder JR. 1980. Plant hormones and other growth substances – their background, structures and occurrence. In: MacMillan J, ed. *Hormonal regulation of development I. Molecular aspects of plant hormones. Encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 9–112.

Beligni MV, Lamattina L. 2001. Nitric oxide in plants: the history is just the beginning. *Plant, Cell & Environment* **24**: 267–278.

Bennet-Clark TA. 1956. The kinetics of auxin-induced growth. In: Wain RL, Wightman F, eds. *Chemistry and mode of action of plant growth substances*. London, UK: Butterworths, 310–312.

Birch MC. 1974. *Pheromones*. New York, USA: North-Holland.

Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. *Annual Review of Cell and Developmental Biology* **16**: 1–18.

Bonner J. 1954. The hormonal control of plant growth. *Harvey Lectures* **48**: 1–34.

Borel C, Frey A, Marion-Poll A, Tardieu F, Siminneau T. 2001. Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? *Plant, Cell & Environment* **24**: 477–489.

Boysen-Jensen P. 1913. Über die Leitung des Phototrophischen Reizes in der Avena-Koleoptile. *Berichte der Deutschen Botanischen Gesellschaft* **31**: 559–566.

Bradford KJ, Trewavas AJ. 1994. Sensitivity thresholds and variable time scales in plant hormone action. *Plant Physiology* **105**: 1029–1036.

Canny MJ. 1984. Translocation of nutrients and hormones. In: Wilkins MB, ed. *Advanced Plant Physiology*. London, UK: Pitman Publishing, 277–296.

Chandler PM, Robertson M. 1999. Gibberellin dose–response curves and the characterization of dwarf mutants of barley. *Plant Physiology* **120**: 623–632.

Cholodny NG. 1927. Wuchshormone und Tropismen bei Den Pflanzen. *Biologisches Zentralblatt* **47**: 604–626.

Clark AJ. 1933. *Mode of action of drugs on cells*. London, UK: Arnold.

Cleland RE. 1972. The dosage-response curve for auxin-induced cell elongation: a re-evaluation. *Planta* **104**: 1–9.

Cleland RE. 1983. Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? – Changes in hormone concentration are important too. *Trends in Biochemical Sciences* **8**: 354–357.

Cornforth JW, Milborrow BV, Ryback G. 1965. Synthesis of (±)-abscisin II. *Nature* **206**: 715.

Cowan AK, Raven JA, Hartung W, Farquhar GD. 1982. A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Australian Journal of Plant Physiology* **9**: 489–498.

Cram WJ. 1976. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Lüttge U, Pitman MG, eds. *Transport in plants IIa. Cells. Encyclopedia of plant physiology, new series, vol. IIA*. Berlin, Germany: Springer Verlag, 284–316.

Creelman RA, Mullet JE. 1997. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 355–381.

Crozier A, Kaimiya Y, Bishop GJ, Yokota T. 2000. The synthesis of hormones and elicitor molecules. In: Buchanan RB, Gruissem W, Jones R, eds. *Biochemistry and molecular biology of plants*. Rockville, MD, USA: American Society of Plant Physiologists. 850–929.

Curtis PJ, Cross BE. 1954. Gibberellic acid. A new metabolite from culture filtrates of *Giberella fujikuroi*. *Chemistry and Industry* **1954, No. 35**: 1066.

Dacie JV, Lewis SM. 1995. *Practical Haematology, 8th edn*. Edinburgh, UK: Churchill Livingston.

Darwin C, Darwin G. 1880. *The power of movement in plants*. New York, USA: Appleton-Century Inc.

Davies E. 1987. Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses. *Plant, Cell & Environment* **10**: 623–631.

Davies WJ, Jones HG. 1991. *Abscisic acid: physiology and biochemistry*. Cambridge, UK: BIOS Scientific Publishers Ltd.

Davies WJ, Wilson JA, Sharp RE, Osonubi O. 1981. Control of stomatal behaviour in water-stressed plants. In: Jarvis PG, Mansfield TA, eds. *Stomatal physiology*. Cambridge, UK: Cambridge University Press, 163–185.

Derkx MPM, Karssen CM. 1993. Changing sensitivity to light and nitrate but not to gibberellins regulates seasonal dormancy patterns in *Sisymbrium officinale* seeds. *Plant, Cell & Environment* **16**: 469–479.

Digby J, Firn RD. 1981. A critical assessment of the Cholodny-Went theory of shoot geotropism. In: Smith H, ed. *Commentaries in plant science, Vol. 2*. Oxford, UK: Pergamon Press, 41–50.

Drury RE. 1969. Interaction of plant hormones. *Science* **164**: 564–565.

Edlemann HG. 2001. Lateral redistribution of auxin is not a means for gravitropic differential growth of coleoptiles: a new model. *Physiologia Plantarum* **112**: 119–126.

Ehlers K, Kollmann R. 2001. Primary and secondary plasmodesmata: structure, origin, and functioning. *Protoplasma* **216**: 1–30.

Esau K. 1965. *Plant anatomy, 2nd edn*. New York, USA: John Wiley & Sons Inc.

Eschrich W. 1975. Sealing systems in phloem. In: Zimmermann MH, Milburn JA, eds. *Transport in plants I. Phloem transport. Encyclopaedia of plant physiology, vol. 1*. Berlin, Germany: Springer-Verlag, 39–56.

Evans ML. 1992. What remains of the Cholodny–Went theory? *Plant, Cell & Environment* **15**: 767–768.

Farmer EE, Ryan CA. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors. *Proceedings of the National Academy of Sciences, USA* **87**: 7713–7716.

Firn RD. 1986. Growth substance sensitivity: the need for clearer ideas, precise terms and purposeful experiments. *Physiologia Plantarum* **67**: 267–272.

Firn RD. 1992. What remains of the Cholodny–Went theory? Which one? *Plant, Cell & Environment* **15**: 769–770.

Firn RD, Digby J. 1980. The establishment of tropic curvatures in plants. *Annual Review of Plant Physiology* **31**: 131–148.

Firn RD, Myers AB. 1987. Hormones and plant tropisms – the degeneration of a model of hormonal control. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 251–261.

Fitting H. 1909. Die Beeinflussing der Orchideenblüten Durch die Bestäubung und Durch Andere Umstände. *Zeitschrift für Botanik* **1**: 1–86.

Fitting H. 1910. Weitere Entwicklungsphysiologische Untersuchtungen an Orchideenblüten. *Zeitschrift für Botanik* **2**: 225–267.

Fitzsimons PJ. 1989. The determination of sensitivity parameters for auxin-induced H^+-efflux from *Avena* coleoptile segments. *Plant Cell & Environment* **12**: 737–746.

Fitzsimons PJ, Barnwell P, Cobb AH. 1988. *A study of auxin-type herbicide action based on dose–response analysis of H^+-efflux. Proceedings of the European Weed Research Society 1988*. Wageningen, The Netherlands: European Weed Reserach Society, 63–68.

Folkers K, Litterru GP, Ho L, Runge TM, Havanonda S, Cooley D. 1970. Evidence for a deficiency of co-enzyme Q10 in human heart disease. *International Journal of Vitamin and Nutritional Research* **40**: 380.

Foster RJ, McRae DH, Bonner J. 1952. Auxin induced growth inhibition, a natural consequence of two-point attachment. *Proceedings of the National Academy of Sciences, USA* **38**: 1014–1022.

Gane R. 1934. Production of ethylene by some ripening fruits. *Nature* **134**: 1008.

Goldsmith M-HM. 1969. Transport of plant growth regulators. In: Wilkins MB, ed. *Physiology of plant growth and development*. Maidenhead, UK: McGraw-Hill, 127–162.

Gove PB. 1961. Ed. *Webster's 3rd New International Dictionary of the English Language, unabridged*. London, UK: Bell & Sons.

Grove MD, Spencer GF, Rohwedder WK, Mandava NB, Worley JF. 1979. Brassionlide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**: 216–217.

Grundvald C. 1980. Steroids. In: Bell EA, Charlewood BV, eds. *Secondary plant products. Encyclopedia of plant physiology, new series, vol. 8*. Berlin, Germany: Springer-Verlag, 221–256.

Haagan-Smit AJ, Leach WD, Bergren WR. 1942. The estimation, isolation and identificatin of auxins in plant tissues. *American Journal of Botany* **29**: 500–506.

Haga K, Uino M. 1998. Auxin-growth relationships in maize coleoptiles and pea internodes and control by auxin of the tissue sensitivity to auxin. *Plant Physiology* **117**: 1473–1486.

Hanson JB, Trewavas AJ. 1982. Regulation of plant cell growth: the changing perspective. *New Phytologist* **90**: 1–18.

Hardie DG. 1999. Plant protein serine/threonine kinases: classification and functions. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 97–131.

Hartung W. 1983. The site of action of abscisic acid at the guard cell plasmalemma of *Valeriana locusta*. *Plant, Cell & Environment* **6**: 427–429.

Hartung W, Radin JW, Hendrix D. 1988. Abscisic acid movement into the apoplastic solution of water stressed cotton leaves: role of apoplastic pH. *Plant Physiology* **86**: 908–913.

Hartung W, Slovik S. 1991. Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid in leaves. *New Phytologist* **119**: 361–382.

Hartung W, Wilkinson S, Davies WJ. 1998. Factors that regulate abscisic acid concentration at the primary site of action at the guard cell. *Journal of Experimental Botany* **49**: 361–367.

Hawkins MB, Thornton JW, Crews D, Skipper JK, Dotte A, Thomas P. 2000. Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleosts. *Proceedings of the National Academy of Sciences, USA* **97**: 10751–10756.

Hillman JR. 1978. *Isolation of plant growth substances*. Cambridge, UK: Cambridge University Press.

Hoad GV, Lenton JR, Jackson MB, Atkin RK. 1987. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths.

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM. 1998. EIN4 and ERS2 are members of the putative ethylene receptor gene family in *Arabidopsis*. *Plant Cell* **10**: 1321–1332.

Huxley JS. 1935. Chemical regulation and the hormone concept. *Biological Reviews* **10**: 427–441.

Iino M. 1992. What remains of the Cholodny–Went theory? Lateral auxin translocation as a key step mediating light-gradient perception and phototropic differential growth. *Plant, Cell & Environment* **15**: 773–774.

Jackson MB. 1985. Ethylene and responses of plants to soil waterlogging and submergence. *Annual Review of Plant Physiology*. **36**: 145–174.

Jackson MB. 1987. A structured evaluation of the involvement of ethylene and abscisic acid in plant responses to aeration stress. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 189–216.

Jackson MB. 1993. Are plant hormones involved in root to shoot communication? *Advances in Botanical Research* **19**: 103–187.

Jacobs WP. 1959. What substance normally controls a given biological process? 1. Formulation of Some Rules. *Developmental Biology* **1**: 527–533.

Jacobs WP. 1979. *Plant hormones and plant development*. Cambridge, UK: Cambridge University Press.

Jenkins GI. 1999. Signal transduction networks and the integration of responses to environmental stimuli. *Advances in Botanical Research* **29**: 53–73.

Jennings DH, Trewavas AJ. 1986. *Plasticity in plants*. Cambridge, UK: Company of Biologists.

Jones RL. 1973. Gibberellins: their physiological role. *Annual Review of Plant Physiology* **24**: 571–598.

Jones HG. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell & Environment* **8**: 95–104.

Jones CA, Firn RD. 1978. The role of phytoecdysteroids in bracken fern *Pterdium aquilinum* (L.) Kühn as a defense against phytophagous insects. *Journal of Chemical Ecology* **14**: 117–138.

Kacser H, Burns JA. 1979. Molecular democracy: who shares the controls? *Biochemical Society Transactions* **7**: 1149–1160.

Kaldewey H. 1984. Transport and other modes of movements of hormones (mainly auxins). In: Scott TK, ed. *Hormonal regulation of development II. The function of hormones from the level of the cell the whole plant. Encyclopedia of plant physiology, new series, vol. 10.* Berlin, Germany: Springer-Verlag, 80–148.

Kappers IF, Jordi W, Maas FM, Stoopen GM, van der Plas L. 1998. Gibberellin and phytochrome control senescence in alstromeria independently. *Physiologia Plantarum* **103**: 91–98.

Karban R, Agrawal AA, Mangel M. 1997. The benefits of induced defences against herbivores. *Ecology* **78**: 1351–1355.

Karssen CM, Laçka E. 1986. A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of *Arabidopsis thaliana*. In: Bopp M, ed. *Plant growth substances 1985*. Berlin, Germany: Springer-Verlag, 315–323.

Keen NT. 1999. Plant disease resistance: progress in basic understanding and practical application. *Advances in Botanical Research* **30**: 291–328.

Kende H. 2001. Hormone response mutants. A plethora of surprises. *Plant Physiology* **125**: 81–81.

Kende H, Gardner G. 1976. Hormone binding in plants. *Annual Review of Plant Physiology.* **27**: 267–290.

Ketterson ED, Nolan V Jr. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* **154**: 4–25.

King RW. 1976. Implications for plant growth of the transport of regulatory compounds in phloem and xylem. In: Wardlaw IF, Passioura JD, eds. *Transport and transfer processes in plants*. New York, USA: Academic Press, 415–431.

Kombrink E, Somssich IED. 1995. Defence responses of plants to pathogens. *Advances in Botanical Research* **21**: 1–34.

Koshland DE, Goldbeter A, Stock JB. 1982. Amplification and adaptation in regulatory and sensory systems. *Science* **217**: 220–225.

Lancaster JE, Mann JD, Porter NG. 1977. Ineffectiveness of abscisic acid in stomatal closure of yellow lupin, *Lupinus luteus* var. Weiko III. *Journal of Experimental Botany* **28**: 184–191.

Larsen P. 1955. Nomenclature of plant growth substances. *Plant Physiology* **30**: 190–191.

Lashbrook CC, Tieman D, Klee HJ. 1998. Differential regulation of the tomato ETR gene family throughout plant development. *Plant Journal* **15**: 243–252.

Leopold AC. 1987. Contemplations on hormones as biological regulators. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 3–15.

Leopold AC, Noodén LD. 1984. Hormonal regulatory systems in plants. In: Scott TK, ed. *Hormonal regulation of development II. The function of hormones from the level of the cell the whole plant. Encyclopedia of plant physiology, new series, vol. 10*. Berlin, Germany: Springer-Verlag, 4–79.

Letham DS. 1963. Zeatin, a factor inducing cell division from *Zea mays. Life Sciences* **8**: 569–573.

Letham DS, Goodwin PB, Higgins TJV. 1978. *Phytohormones and related compounds: a comprehensive treatise*. Amsterdam, The Netherlands: Elsevier North-Holland Biomedical Press.

Leung J, Giraudat J. 1998. Abscisic acid signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 199–222.

Loeb JN, Strickland S. 1987. Hormone binding and coupled response relationships in systems dependent on the generation of secondary mediators. *Molecular Endocrinology* **1**: 75–82.

Löve A, Löve D. 1945. Experiments on the effects of animal sex hormones on dioecious plants. *Arkives für Botanik* **32**: 1–60.

MacDonald IR, Hart JW. 1987. New light on the Cholodny-Went theory. *Plant Physiology* **84**: 568–570.

MacDowall FDH, Sirois JC. 1977. Importance of time after excision and pH on the kinetics of response of wheat coleoptile segments to added indole acetic acid. *Plant Physiology* **59**: 405–410.

MacMillan J. 1980. Hormonal regulation of development. 1. Molecular aspects of plant hormones. *Encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 219–243.

MacMillan J. 1987. Gibberellin-deficient mutants of maize and pea and the molecular action of gibberellins. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 73–87.

MacMillan J, Suter PJ. 1958. The occurrence of gibberellin A_1 in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). *Naturwissenschaften* **45**: 46.

MacRobbie E. 1991. Effect of ABA on ion transport and stomatal regulation. In: **Davies WJ, Jones HG**, eds. *Abscisic acid physiology and biochemistry*. Oxford, UK: BIOS Scientific Publishers, 153–168.

Malone M. 1996. Rapid, long-distance signal transmission in plants. *Advances in Botanical Research* **22**: 163–228.

Matthysse AG, Scott TK. 1984. Functions of hormones at the whole plant level of organization. In: Scott TK, ed. *Hormonal regulation of development II. The function of hormones from the level of the cell to the whole plant. Encyclopedia of plant physiology, new series, vol. 10*. Berlin, Germany: Springer-Verlag, 219–243.

Mayer AM, Poljakoff-Mayber A. 1982. *The germination of seeds, 3rd edn.* Oxford, UK: Pergamon Press.

McAinsh MR, Brownlee C, Sarsag M, Webb AAR, Hetherington AM. 1991. Involvement of second messengers in the action of ABA. In: Davies WJ, Jones HG, eds. *Abscisic acid physiology and biochemistry*. Oxford, UK: BIOS Scientific Publishers, 137–152.

McAinsh MR, Gray JE, Hetherington AM, Leckie CP, Ng C. 2000. Ca^{2+} signalling in stomatal guard cells. *Biochemical Society Transations* **28**: 476–481.

McCourt P. 1999. Genetic analysis of hormone signalling. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 219–243.

McGlasson WB, Pratt HK. 1964. Effect of ethylene on cantaloupe fruits harvested at different ages. *Plant Physiology* **39**: 120–127.

McIntyre GI. 2001. Control of plant development by limiting factors: a nutritional perspective. *Physiologia Plantarum* **113**: 165–175.

Meidner H. 1985. Historical sketches 6. *Journal of Experimental Botany* **36**: 1017–1018.

Mer C. 1968. What is an inhibitor? *Zeischrift für Pflanzenphysiologie* **59**: 415–419.

Meyerowitz EM. 1999. Plants, animals and the logic of development. *Trends in Biochemical Sciences* **24**: 65–68.

Milborrow BV. 1974. The chemistry and physiology of abscisic acid. *Annual Review of Plant Physiology* **25**: 259–307.

Mohr H, Schopfer P. 1994. *Plant physiology*. New York, USA: Springer-Verlag.

Moore TC. 1989. *Biochemistry and Physiology of Plant Hormones, 2nd edn.* New York, USA: Springer-Verlag.

Morgan PH, Mercer LP, Flodin NM. 1975. General model for nutritional responses of higher organisms. *Proceedings of the National Academy of Sciences, USA.* **72**: 4327–4331.

Morton RA. 1974. The vitamin concept. *Vitamins and Hormones* **32**: 155–166.

Mulkey TJ, Kuzmanoff KM, Evans ML. 1982. Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors aminoethoxyvinylglycine and cobalt. *Plant Science Letters* **25**: 43–48.

Munns R, King RW. 1988. Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plants. *Plant Physiology* **88**: 703–708.

Newman EI. 1983. Interactions between plants. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. Physiological plant ecology III. Responses to the chemical and biological environment. *Encyclopedia of plant physiology, new series, vol. 12c*. Berlin, Germany: Springer-Verlag, 697–710.

Ni BR, Bradford KJ. 1993. Germination and dormancy of abscisic-acid and gibberellin deficient mutant tomato seeds. Sensitivity of germination to abscisic acid, gibberellin and water potential. *Plant Physiology* **101**: 607–617.

Nissen P. 1985. Dose–responses of auxins. *Physiologia Plantarum* **65**: 357–374.

Nissen P. 1988a. Dose–responses of gibberellins. *Physiologia Plantarum* **72**: 197–203.

Nissen P. 1988b. Dose–responses of cytokinins. *Physiologia Plantarum* **74**: 450–456.

Nissl D, Zenk MH. 1969. Evidence against induction of protein synthesis during auxin-induced initial elongation of *Avena* coleoptiles. *Planta* **89**: 323–341.

Nobel PS. 1991. *Physicochemical and environmental plant physiology*. San Diego, CA, USA: Academic Press, Inc.

O'Donnell PJ, Jones JB, Antoine FR, Ciardi J, Klee HJ. 2001. Ethylene-dependent salicylic acid regulates an expanded cell death response to a plant pathogen. *Plant Journal* **25**: 315–323.

Ogunkanmi AB, Tucker DJ, Mansfield TA. 1973. An improved bioassay for abscisic acid and other antitranspirants. *New Phytologist* **72**: 277–282.

Oparka KJ, SantaCruz S. 2000. The great escape: phloem transport and the unloading of macromolecules. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**: 323–347.

Orton PJ, Mansfield TA. 1974. The activity of abscisic acid analogues as inhibitors of stomatal opening. *Planta* **121**: 263–272.

Osborne DJ. 1977. Ethylene and target cells in the growth of plants. *Science Progress* **64**: 53–65.

Paál A. 1919. Über Phototropische Reizleitung. *Jahrbuch für Wissenschaften Botanik* **58**: 406–458.

Parsons R, Stanforth A, Raven J, Sprent JI. 1993. Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. *Plant, Cell & Environment* **16**: 125–136.

Pate JS. 1976. Nutrients and metabolites of fluids recovered from xylem and phloem: significance in relation to long-distance transport in plants. In: Wardlaw IF, Passioura JD, eds. *Transport and transfer processes in plants.* New York, USA: Academic Press, 253–281.

Paterson NW. 1989. *Hormonal sensitivity and stomatal movement.* PhD thesis. Dundee, UK: University of Dundee.

Paterson NW, Herdman L, Weyers JDB. 2001. Relative control potential of abscisic acid, carbon dioxide and light in responses of *Phaseolus vulgaris* stomata. *Physiologia Plantarum* **111**: 412–418.

Paterson NW, Weyers JDB, A'Brook R. 1988. The effect of pH on stomatal sensitivity to abscisic acid. *Plant, Cell & Environment* **11**: 83–89.

Paterson NW, Weyers JDB, A'Brook R, Fitzsimons PJ. 1990. Plant hormone sensitivity. In: Purohit SS, ed. *Hormonal regulation of plant growth and development, vol. V*. Bikaner, India: Agro Botanical Publishers, 61–90.

Patten BC. 1976. *Systems analyses and simulation in ecology, vol. 4*. New York, USA: Academic Press.

Pearce G, Strydom D, Johnson S, Ryan CA. 1991. A polypeptide from tomato leaves activates the expression of proteinase inhibitor genes. *Science* **253**: 895–887.

Peng Z-Y, Weyers JDB. 1994. Stomatal sensitivity to abscisic acid following water deficit stress. *Journal of Experimental Botany* **45**: 835–845.

Peuke AD, Rokitta M, Zimermann U, Schreiber L, Haase A. 2001. Simultaneous measurement of water flow velocity and solute transport in xylem and phloem of adult plants of *Ricinus communis* over a daily time course by nuclear resonance spectrometry. *Plant, Cell & Environment* **24**: 491–503.

Phillips IDJ. 1971. *Introduction to the biochemistry and physiology of plant growth hormones*. New York, USA: McGraw-Hill Book Co.

Phinney BO. 1984. Gibberellin A_1 dwarfism and the control of shoot elongation in higher plants. In: Crozier A, Hillman JR, eds. *The biosynthesis and metabolism of plant hormones*. Cambridge, UK: Cambridge University Press, 17–41.

Pierpoint WS. 1994. Salicylic acid and its derivitives in plants: medicines, metabolites and messenger molecules. *Advances in Botanical Research* **20**: 163–235.

Pincus G, Thimann K. 1948. *The hormones*. New York, USA: Academic Press.

Radin JW. 1984. Stomatal responses to water stress and abscisic acid in phosphorous-deficient cotton plants. *Plant Physiology* **76**: 392–394.

Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiology* **67**: 115–119.

Radin JW, Ackerson RC. 1982. Does abscisic acid control stomatal closure during water stress? *What's New in Plant Physiology* **12**: 9–12.

Radin JW, Hartung W, Kimball BA, Mauney JR. 1988. Correlation of stomatal conductance with photosynthetic capacity of cotton only in a CO_2-enriched atmosphere: mediation by abscisic acid? *Plant Physiology* **88**: 1058–1062.

Raschke K. 1979. Movement of stomata. In: Haupt W, Feinleib ME, eds. *Physiology of movements. Encyclopaedia of plant physiology, new series, vol. 7*. Berlin, Germany: Springer-Verlag, 383–411.

Reeve DR, Crozier A. 1980. Quantitative analysis of plant hormones. In: MacMillan J, ed. *Hormonal regulation of development I. Molecular aspects of plant hormones. encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 203–280.

Rice EL. 1984. *Allelopathy*. New York, USA: Academic Press.

Rizvi SJH, Haque H, Singh VK, Rizvi V. 1992. A discipline called allelopathy. In: Rizvi SJH, Rizvi V, eds. *Allelopathy: basic and applied aspects*. London, UK: Chapman & Hall, 1–10.

Roberts JA, Hooley R. 1988. *Plant growth regulators*. Glasgow, UK: Blackie.

Rodriguez JL, Davies WJ. 1982. The effect of temperature and ABA on stomata of *Zea mays* L. *Journal of Experimental Botany* **33**: 977–987.

Ross J, O'Neill D. 2001. New interactions between classical plant hormones. *Trends in Plant Science* **6**: 2–4.

Ryan CA, Pearce G. 2001. Polypeptide hormones. *Plant Physiology* **125**: 65–68.

Sack FD. 1981. The development and structure of stomata. In: Zeiger E, Farquhar GD, Cowan IR, eds. *Stomatal function*. Stanford, CA, USA: Stanford University Press, 59–89.

Salisbury FB. 1992. What remains of the Cholodny-Went theory? A potential role for changing sensitivity to auxin. *Plant, Cell & Environment* **15**: 785–786.

Salisbury FB, Gillespie L, Rorabaugh P. 1988. Gravitropism in higher plant shoots V. Changing sensitivity to auxin. *Plant Physiology* **88**: 1186–1194.

Salisbury FB, Ross CW. 1992. *Plant physiology, 4th edn*. Belmont, CA, USA: Wadsworth Publishing Co.

Sasse JM. 1990. Brassinolide-induced elongation and auxin. *Physiologia Plantarum* **80**: 401–408.

Schistad IJ, Nissen P. 1984. Cytokinin-induced retention of chlorophyll in senescing barley leaves: complexity of dose–response. *Physiologia Plantarum* **61**: 566–570.

Schnabl H, Bornman CH, Zeigler H. 1978. Studies of isolated starch-containing (*Vicia faba*) and starch-deficient (*Allium cepa*) guard cell protoplasts. *Planta* **143**: 33–40.

Scott TK. 1984. Hormonal regulation of development II. The function of hormones from the level of the cell tothe whole plant. In: Scott TK, Evans ML, eds. *Encyclopedia of plant physiology, new series, vol. 10*. Berlin, Germany: Springer-Verlag.

Sembdner G, Gross D, Leibisch H-W, Schneider G. 1980. Biosynthesis and metabolism of plant hormones. In: MacMillan J, ed. *Hormonal regulation of development I. Molecular aspects of plant hormones. Encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 281–444.

Shulalev V, Silverman P, Raskin I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* **385**: 718–721.

Smalle J, Van der Straeten D. 1997. Ethylene and vegetative development. *Physiologia Plantarum* **100**: 593–605.

Smith TA. 1985. Polyamines. *Annual Review of Plant Physiology* **36**: 117–143.

Spanswick RM. 1972. Electrical coupling between cells of higher plants: a direct demonstration of intercellular communication. *Planta* **102**: 215–227.

Starling EH. 1905. The chemical correlations of the function of the body. *Lancet* **2**: 339–341.

Steudle H. 2001. The cohesion-tension mechanism and the acquisition of water by plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 847–875.

Steward FC. 1960. *Plant physiology: a treatise*. New York, USA: Academic Press.

Stoddart JL, Venis MA. 1980. Molecular and subcellular aspects of hormone action. In: MacMillan J, ed. *Hormonal regulation of development I. Molecular aspects of plant hormones. Encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 445–510.

Strickland S, Loeb JN. 1981. Obligatory separation of hormone binding and biological response curves in systems dependent upon secondary mediators of hormone action. *Proceedings of the National Academy of Sciences, USA* **78**: 1366–1370.

Swarup R, Marchant A, Bennett MJ. 2000. Auxin transport: providing a sense of direction during plant development. *Biochemical Society Transactions* **28**: 481–485.

Swift S, Throup JP, Williams P, Salmond GPC, Stewart GSAB. 1996. Quorum sensing: a population-density component in the determination of bacterial phenotype. *Trends in Biochemistry* **21**: 214–219.

Takahashi N. 1986. *Chemistry of plant hormones*. Boca Raton, FL, USA: CRC Press.

Takahashi N, Yamaguchi I, Yamane H. 1986. Gibberellins. In: Takahashe N, ed. *Chemistry of plant hormones*. Boca Raton, FL, USA: CRC Press, 57–151.

The *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–815.

Thimann K. 1948. Plant Growth Hormones. In: Pincus G, Thimann K, eds. *The hormones, vol. 1*. New York, USA: Academic Press.

Thomas H, Grierson D. 1987. *Developmental mutants in higher plants*. Cambridge, UK: Cambridge University Press.

Tieman DM, Klee HJ. 1999. Differential expression of two novel members of the tomato ethylene receptor family. *Plant Physiology* **120**: 165–172.

Trejo CL, Clephan AL, Davies WJ. 1995. How do stomata read abscisic acid signals? *Plant Physiology* **109**: 803–811.

Trewavas AJ. 1981. How do plant growth substances work? *Plant, Cell & Environment* **4**: 203–228.

Trewavas AJ. 1982. Growth substance sensitivity: the limiting factor in plant development. *Physiologia Plantarum* **35**: 60–72.

Trewavas AJ. 1983a. Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? – Sensitivity is the regulating factor. *Trends in Biochemical Sciences* **8**: 354–357.

Trewavas AJ. 1983b. Plant growth substances – metabolic flywheels for plant development. *Cell Biology International Reports* **7**: 569–575.

Trewavas AJ. 1983c. Nitrate as a plant hormone. In: Jackson MB, ed. *Interactions between nitrogen and growth regulators in plant development*. Oxford, UK: British Plant Growth Regulator Group, 97–110.

Trewavas AJ. 1986a. Resource allocation under poor growth conditions. A major role for growth substances in developmental plasticity. In: Jennings DH, Trewavas AJ, eds. *Plasticity in plants*. Cambridge, UK: Company of Biologists, 31–76.

Trewavas AJ. 1986b. Understanding the control of plant development and the role of growth substances. *Australian Journal of Plant Physiology* **13**: 447–457.

Trewavas AJ. 1987. Sensitivity and sensory adaptation in growth substance responses. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK, eds. *Hormone action in plant development – a critical appraisal*. London, UK: Butterworths, 19–38.

Trewavas AJ. 1991. How do plant growth substances work? II. *Plant Cell & Environment* **14**: 1–12.

Trewavas AJ. 1992. What remains of the Cholodny-Went theory? A summing up. *Plant, Cell & Environment* **15**: 793–794.

Trewavas AJ, Jones AM. 1981. Consequences of hormone-binding studies for plant growth substance research. *What's New in Plant Physiology* **12**: 5–8.

Trewavas AJ, Jones HG. 1991. An assessment of the role of ABA in plant development. In: Davies WJ, Jones HG, eds. *Abscisic acid: physiology and biochemistry*. Cambridge, UK: BIOS Scientific Publishers, 169–188.

Tukey HB, Went FW, Muir RM, Van Overbeek J. 1954. Nomenclature of chemical plant regulators. *Plant Physiology* **29**: 307–308.

Ueda J, Kato J. 1980. Isolation and identification of a senescence-promoting substance from wormwood *Artemisia absinthium* L. *Plant Physiology* **66**: 246–249.

Venis MA. 1985. *Hormone binding sites in plants*. London, UK: Longman.

Venis MA, Thomas EW, Barbier-Brygoo H, Ephritikhine G, Guern J. 1990. Impermeant auxin analogues have auxin activity. *Planta* **182**: 232–235.

Voesenek LACJ, Banga M, Rijnders JGHM, Visser EJW, Blom CWPM. 1996. Hormone sensitivity and plant adaptations to flooding. *Folia Geobotanica and Phytotaxonomica* **31**: 47–56.

Wareing PF. 1977. Growth substances and integration in plants. In: Jennings DH, ed. *Integration of activity in the higher plant*. Cambridge, UK: Cambridge University Press, 337–365.

Webb AAR, McAinsh MR, Taylor JE, Hetherington AM. 1996. Calcium ions as intracellular second messengers in higher plants. *Advances in Botanical Research* **22**: 45–96.

Weiler EW, Eberle J, Merten R, Atzorn R, Feyerabend M, Jourdan PS, Arnscheidt A, Wieczorek U. 1986. Antisera- and monoclonal antibody-based immunoassay of plant hormones. In: Wang TL, ed. *Immunology in plant science*. Cambridge, UK: Cambridge University Press, 27–58.

Went FW, Thimann KV. 1937. *Phytohormones*. New York: The MacMillan Co.

Weyers JDB. 1994. Do plants *really* have hormones? *New Scientist* **102**: 9–13.

Weyers JDB, Hillman JR. 1979a. Sensitivity of *Commelina* stomata to abscisic acid. *Planta* **146**: 623–628.

Weyers JDB, Hillman JR. 1979b. Uptake and distribution of abscisic acid in *Commelina* leaf epidermis. *Planta* **144**: 167–172.

Weyers JDB, Lawson T. 1997. Heterogeneity in stomatal characteristics. *Advances in Botanical Research* **26**: 317–352.

Weyers JDB, Meidner H. 1990. *Methods in stomatal research*. Harlow, UK: Longman Scientific and Technical.

Weyers JDB, Paterson NW. 1992. Quantitative assessment of plant hormone sensitivity with reference to stomatal responses to abscisic acid. In: Karssen CM, van Loon LC, Vreugdenhil D, eds. *Progress in plant growth regulation*. Dordecht, The Netherlands: Kluwer Academic Publishers, 226–236.

Weyers JDB, Paterson NW, A'Brook R. 1987. Towards a quantitative definition of plant hormone sensitivity. *Plant, Cell & Environment* **10**: 1–10.

Weyers JDB, Paterson NW, A'Brook R, Peng Z-Y. 1995. Quantitative analysis of the control of physiological phenomena by plant hormones. *Physiologia Plantarum* **95**: 486–494.

Weyers JDB, Peng Z-Y. 1995. The role of abscisic acid in the control of stomatal movements during water deficit stress. In: Purohit SS, ed. *Agro's annual review of plant physiology (basic and applied), vol. 2*. Bikaner, India: Agro Botanical Publishers, 205–236.

Wheeler RM, Salisbury FB. 1981. Gravitropism in higher plant shoots I. A role for ethylene. *Plant Physiology* **67**: 686–690.

Wilkins MB. 1984. Gravitropism. In: Wilkins MB, ed. *Advanced plant physiology*. London, UK: Pitman Publishing, 163–185.

Wilkinson S, Corlett JE, Oger L, Davies WJ. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves. *Plant Physiology* **117**: 703–709.

Williamson GB. 1990. Allelopathy, Koch's postulates and the neck riddle. In: Grace JB, Timan D, eds. *Perspectives on plant competition*. San Diego, CA, USA: Academic Press, 143–162.

Willmer CM, Fricker M. 1996. *Stomata, 2nd edn*. London, UK: Chapman & Hall.

Wright STC. 1966. Growth and cellular differentiation in the wheat coleoptile (*Triticum vulgare*) II. Factors influencing the growth response to gibberellic acid, kinetin and indole-3-acetic acid. *Journal of Experimental Botany* **17**: 165–176.

Yamamura N, Tsuji N. 1995. Optimal strategy of plant antiherbivore defense – implications for apparency and resource-availability theories. *Ecological Research* **10**: 19–30.

Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* **35**: 155–189.

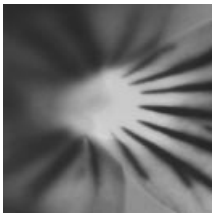
Yokata T, Murofushi N, Takahashi N. 1980. Extraction, purification and identification. In: MacMillan J, ed. *Hormonal regulation of development I. Molecular aspects of plant hormones. Encyclopedia of plant physiology, new series, vol. 9*. Berlin, Germany: Springer-Verlag, 113–201.

Zhang SQ, Outlaw WH. 2001. The guard-cell apoplast as the site of abscisic acid accumulation in *Vicia faba* L. *Plant, Cell & Environment* **24**: 347–355.

Zhang J, Schurr U, Davies WJ. 1987. Control of stomatal behaviour by abscisic acid which apparently originates in the roots. *Journal of Experimental Botany* **38**: 1174–1181.

Ziegler H. 1975. Nature of transported substances. In: Zimmermann MH, Milburn JA, eds. *Transport in plants I. Phloem transport. Encyclopaedia of plant physiology, vol. 1*. Berlin, Germany: Springer-Verlag, 59–100.

Zucconi F. 1988. Epigenetic regulation in plants. *Israel Journal of Botany* **37**: 131–144.



New Phytologist Symposia

The *New Phytologist* Trust is dedicated to the promotion of plant science. *New Phytologist* Symposia – independent, international meetings – are one way in which the Trust fulfils this aim, supporting the research community in bringing together the top scientists in a given field. These meetings, established in 1995, have quickly gained a reputation for excellence through stimulating presentations and open discussion:

- Plant–microbe symbiosis – molecular approaches
  York, UK, 1995

- Putting plant physiology on the map – genetic analysis of developmental and adaptive traits
  Bangor, UK, 1997

- Major biological issues resulting from anthropogenic disturbance of the nitrogen cycle
  Lancaster, UK, 1997

- At the crossroads of plant physiology and ecology – causes and consequences of variation in leaf structure
  Montpellier, France, 1998

- Root dynamics and global change – an ecosystem perspective
  Tennessee, USA, 1999
  Held in collaboration with GCTE

- Signalling in plants
  Wye, UK, 2000
  Held in collaboration with The Biochemical Society

- Stomata 2001
  Birmingham, UK, 2001

2002 is the 100th Anniversary Year of *New Phytologist* and, celebrating this, the *New Phytologist* Trust will be funding three special symposia in 2002, located in three different countries and covering the three sections of the journal, *Function* (Section Editor Dale Sanders FRS), Environment (Section Editor Richard Norby) and *Interaction* (Section Editor Francis Martin). *New Phytologist* is committed to publishing top research across the breadth of plant science, and it is hoped that these symposia will provide focus to that aim. As usual, the Trust is offering a number of bursaries for those research students and postdoctoral scientists who are presenting posters.

The first of this series of three, and the 8th *New Phytologist* Symposium, will be 'Impacts of soil microbes on plant population dynamics and productivity', to be held at the Viikki Biocenter (Infocenter), University of Helsinki, Finland on 10–14 June 2002. If you are interested in attending, please get in touch with Robin Sen (robin.sen@helsinki.fi).

If you have any other queries about the symposia, do not hesitate to get in touch with Central Office (newphytol@lancaster.ac.uk).