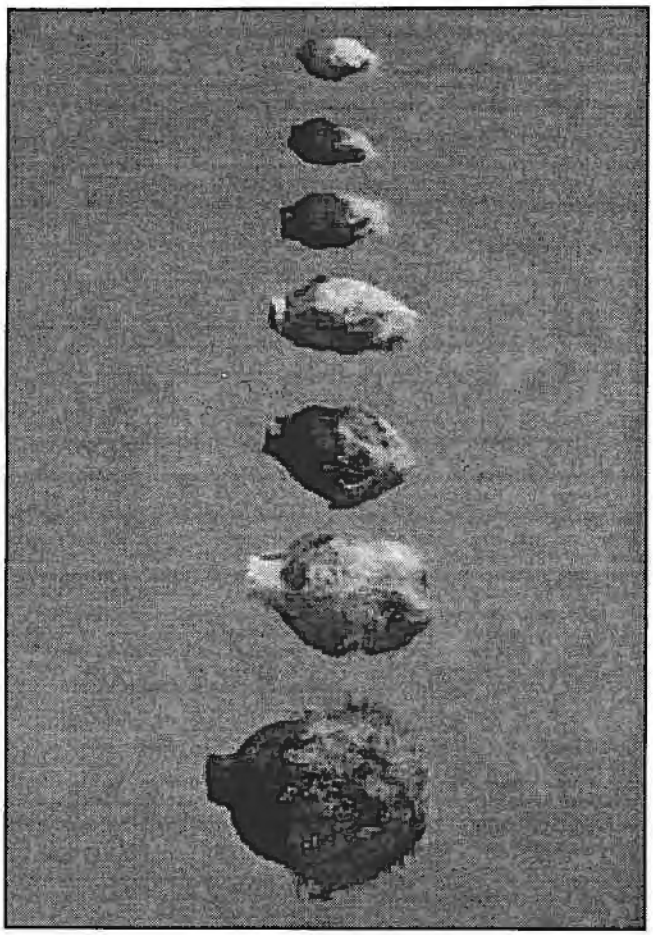
8 *Physical cues for growth and reproduction*



Amongst other fundamental properties, the protoplasm of plants is endowed with that of irritability, a certain sensitiveness, that is, to the influence of external agents.

(Sydney Howard Vines, Lectures on the Physiology of Plants, 1886)

On the one hand, the farmer is concerned with the living plant; on the other, with that complex set of factors we call the environment ... A plant, like an animal, is a sensitive living thing. Plants make responses to their environment [which] ... may be expressed in tons of leaves and stems, in tons of roots, in pounds of seed or grain, in barrels of fruit, or in per cent of sugar, or starch, or acid ... First, we must understand something of the structure and functions of the plant. Second, we must have a knowledge of the various factors of the environment. And, third, we must know the manner in which the plant behaves under a given set of conditions. This is a big order. It is asking much.

(Wilfred W. Robins, Principles of plant growth, 1927)

Adaptation of a temperate plant, peach, to cropping in the sub-tropics. This variety, Flordaking, has been bred with reduced dormancy which confers a 'low chill' requirement. This allows the reproductive cycle to proceed at latitudes (29°S in this instance) where winters are insufficiently cold to break the deeper dormancy of normal 'high chill' varieties. Developing flowers were excised from within the protective bud scales over a period from early autumn (March, left) to mid-winter (July, right) and show continued slow growth throughout (? see Colour Plate xx)
(Photograph courtesy J.J. Lloyd and C.G.N. Turnbull)

Chapter outline

Introduction

Probably since the beginning of civilisation, humans have observed that plants are seasonal organisms. Whether crop plant or native species, annual or perennial, herbaceous or woody, the most obvious manifestation is in time of flowering. The connection of periodic flowering — and subsequently fruit and seed development — with seasonal climates has also been surmised for centuries, but we now know which environmental factors are largely responsible for regulating time of flowering. In this chapter, we focus on the most critical signals, *photoperiod*, *temperature* and *water*. Other signals enable plants to attune themselves for optimum development at other stages of the life cycle: directional stimuli such as *light*, *gravity* and *touch*, as well as dramatic cues for stopping and starting life, namely *fire* and *drought*. Many of these strategies will be highlighted further in Part IV. The chapter concludes with an exploration of how photoreceptors function.

8.1 Latent life: dormancy

8.1.1 Dormancy: the phenomenon of suspended animation

Most plants enter a state of latent life at least once throughout their life cycle. This is dormancy, concisely defined as 'the temporary suspension of visible growth of any plant structure containing a meristem' (Lang 1987). It encompasses a widespread but remarkable phenomenon and is really a collective term covering a number of processes in different plant organs. This has led to problems with terminology, which Lang resolves into three types of dormancy based on their controlling factors:

1. Endodormancy, often called 'true' dormancy, which is the prevention of growth due to factors within a meristem. Failure of a bud to grow in early winter due to insufficient chilling, even if it is exposed to warm conditions, is an example of endodormancy.
2. Paradormancy, which is the suspension of growth caused by factors outside the meristem but within the plant. It is typically an influence of one organ over another, and includes an apical bud preventing outgrowth of a lower bud, which relates to apical dominance (see Martin 1987 for review). Dormancy imposed by factors in the seed coat is, strictly speaking, a version of paradormancy, because the embryo germinates readily when excised from the seed.
3. Ecodormancy, which is the prevention of growth due to environmental conditions such as lack of water or temperature extremes. This is also referred to as quiescence or imposed dormancy (Crabbe 1994).

These definitions are tailored towards woody perennials, but we are also interested in equivalent phenomena in seeds and vegetative storage organs. Indeed there are underlying similarities, for example in endodormancy release induced by chilling. A dormant bud on a perennial contains reduced leaves and floral and/or vegetative meristems, and relies on the rest of the plant for water and nutrients. A storage organ, such as a bulb or tuber, is also a plant propagule containing meristems (Figure 7.17e) and its own reserves of nutrients. Likewise, a seed contains a whole plant — the embryo — and associated storage reserves. Resumption of bud growth leads to shoot emergence through the bud scales, and seed germination results in radicle then shoot emergence through a protective seed coat. These morphological differences may require variations in the physiological control of dormancy.

(a) Why is dormancy important in agriculture?

Plants are generally adapted to their natural environments but many economically important species are cultivated in other climates. Adaptations are genetically based and may be impossible to switch off, or at least difficult to overcome. Temperate fruit trees, such as peaches, eventually become endodormant even in the tropics. Without chilling or human intervention, they do not resume normal growth and may even die. Generally, though, plants will eventually dispense with dormancy-breaking requirements rather than die, often described as a conversion from an obligate to a facultative state. Although tropical perennials cannot tolerate cold temperate winters, they still exhibit endodormancy phases which alternate with dramatic 'flushing' of new vegetative shoots, often with striking red coloured leaves, as in *Syzygium* and mango trees. Dormancy may also prevent or retard seed germination or sprouting of bulbs, thus reducing the number, quality and uniformity of plants in a crop.

8.1.2 Seed dormancy

For most plants, seeds are the primary system of reproduction. Dormancy allows seeds to separate from their mother plant and survive dispersal over distance and time before growth

recommences. Developing embryos are growing tissues but enter dormancy late in maturation and seeds then dehydrate. This state of suspended animation enhances chances of survival. The torpedo-shaped seed of a mangrove (*Rhizophora maritima*) is an exception that germinates while still on the mother plant. When they fall, seed penetrate securely into soft mud flats. This adaptation aids speed of establishment in the unstable tidal zone.

Plant breeders often select seed for uniform, rapid germination but these characteristics are rare in nature. If all seed from a species or population germinated synchronously but was subsequently destroyed, say, by frost, the genome would be lost. Instead, we find that germination is usually staggered over a season or over years. Sometimes it is possible to harvest seeds or embryos before dormancy is induced and thereby germinate otherwise difficult species.

There are two main reasons why a seed does not germinate: it may be dead (not viable) or dormant (Mott and Groves 1981; Langkamp 1987). Vital stains can confirm viability of embryos (Bewley and Black 1982). Embryos may never develop due to post-zygotic incompatibility (Section 7.2.4), may abort during development or may die after seed dispersal. Endodormant or paradormant seed may be viable, but may not germinate even when supplied with water and O_2 at an appropriate temperature.

Seed longevity often relates to a species' natural environment. In climates favourable for germination, many species have seeds which remain viable for only a few days, for example the Queensland umbrella tree (*Schefflera actinophylla*), which originates in subtropical rainforests, or a few months, for example water gum (*Tristania laurina*) and myrtle beech (*Nothofagus cunninghamii*), which come from cooler rainforests. In contrast, seed from sclerophyllous forests, such as *Eucalyptus* and *Acacia* (Figure 8.1), remain viable for many years.

There are two categories of seed, recalcitrant and orthodox, and appropriate storage can vastly extend longevity of both. Many tropical and subtropical species, such as *Citrus*, mango and rambutan, have recalcitrant seeds; these are not desiccation tolerant and survive best if stored at high water content (30%) and warm temperature (usually >15°C). Orthodox seeds, such as *Eucalyptus* and *Brassica*, are usually stored below 10% water content and below 10°C. Between these extremes are many intermediates, and optimum conditions for several important crop species have been determined by empirical experiment. For example, wheat is best stored at 14.5% seed water content, peas at 14.0% and clover at 11.0%.

Cells of some testas have hard, thick walls and a waxy layer which prevents imbibition (uptake of water) and sometimes even gas exchange. Dormancy persists in the absence of water or O_2 essential for germination. Seed-coat-imposed dormancy is a special case closely related to paradormancy of perennials. Seed coats resist embryo expansion but plant tissues can exert substantial turgor pressure, so mechanical resistance is not a common form of dormancy. Roses have a very hard

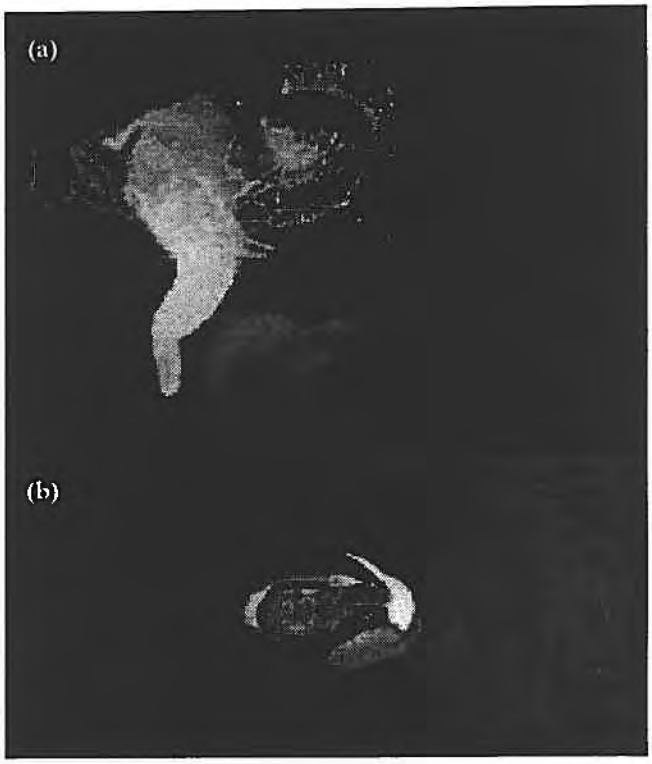


Figure 8.1 Long-lived seeds of species typical of Australian sclerophyll forests. (a) *Eucalyptus erythrocorys* radicle emerging from capsule; (b) *Acacia cochlearis* with fleshy aril still attached
(Photographs courtesy P.T. Austin and J.A. Plummer)

seed coat with several sclerified (stony) cell layers and great pressure is required to break them. Hard seeds are found in many families and are particularly common in legumes such as Fabaceae (e.g. clover (*Trifolium*) and lucerne (*Medicago*)), Mimosaceae (e.g. *Acacia*), and Caesalpiniaceae (e.g. *Cassia*). The seed coat exerts force on the strophiole, a plug-like valve structure near the hilum with elongated malphigian cells that separate to permit water entry. These seed coats need to be weakened physically or chemically to permit imbibition. This may occur naturally as a result of temperature fluctuations, abrasion and microbial or insect damage. Artificial scarification is often achieved by scratching, nicking or by rotating seeds in barrels containing an abrasive. Alternatively, seed can be chemically scarified with concentrated H_2SO_4, which mimics the effect of acid in the stomach of animals. In many parts of Australia spontaneous fire is common and destroys most living tissue but enables germination of many hard-seeded native species (Table 8.1; Bell *et al.* 1993). In these plants, brief seed boiling is commonly substituted to effect break of dormancy. Heat from fires will damage the testa, but smoke, perhaps via ethylene and/or sulphur compounds (Dixon *et al.* 1995), is also effective in overcoming other dormancy mechanisms. In serotinous plants, such as *Hakea*, *Banksia* and *Eucalyptus*, seeds are stored on the mother plant until fires open the woody fruits, dispersing the seeds into the nutrient-rich ash bed when competition for light from other plants has also been reduced.

Germination inhibitors can be present in the embryo, endosperm, testa or the surrounding fruit tissues. Inhibitors

Table 8.1 Fire stimulates germination via several mechanisms: (1) damage to the testa — equivalent to scarification or boiling; (2) reduction of germination inhibitors through heat and leaching; (3) smoke exudates (not all species have evolved with fire); (4) scarification required — but heat from fire kills seed

Species	Type	Fire response	Control	Scarification	Boiling 30 s	Boiling 60 s	Smoke
			Germination (%)				
Acacia nervosa[a]	1	Yes	16[b]	66	72	70	—
Acacia lasiocarpa[a]	2	Yes	20	30	82	82	—
Daviesia preissii[a]	1	Yes	0	55	58	18	—
Gompholobium knightianum[a]	1	Yes	6	51	61	86	6
Conostylis setosa	1,3	Yes	1	0		53	48
Conospermum triplinervum	3	Yes	26				88
Anigozanthus manglesii	3	Yes	3	4		6	32
Lechanaultia biloba	3	Yes	1			0	40
Thysanotus multiflorus	3	Yes	0	0		0	31
Acacia cyclops[a]	4	No	12	72	—	20	—

[a]Legume; [b] = % germination
(Adapted from Bell *et al.* 1993 and Dixon *et al.* 1995)

Table 8.2 Seeds of lettuce cultivar Grand Rapids were exposed to brief periods of alternating red (R = 660 nm) and far red (FR = 720 nm) light. The response depends on the last exposure and is typical of photoreversible phytochrome responses

Treatment	Germination (%)
Darkness	8.5
Red (R)	98
Red→Far Red (FR)	54
R→FR→R	100
R→FR→R→FR	43
R→FR→R→FR→R	99

(From Borthwick et al. 1954)

Table 8.3 In species which usually require periods in dry storage, alternative treatments can be used to break dormancy

Species	Common name	Dry storage period (months)	Alternative treatment
Triticum aestivum	Wheat	3–7	Stratification
Hordeum vulgare	Barley	0.5–9	Stratification, GA_3
Avena fatua	Wild oats	30	stratification, GA_3, ethylene
Oenothera odorata	Evening primrose	7	KNO_3
Impatiens balsamina	Balsam	4–6	stratification
Rumex crispus	Curled dock	60	light, stratification, alternating temperatures
Lactuca sativa	Lettuce cv. Grand Rapids	3–9	light, GA_3, cytokinin stratification

(Adapted, with permission, from Bewley and Black 1994)

present in seed of *Iris*, freshly harvested hazelnut (*Corylus avellana*) and desert ephemerals, and in fleshy fruit such as tomato, *Persoonia* and *Lomandra*, must be removed or inactivated before germination can proceed; this often happens inside an animal gut or by rain leaching.

Many species germinate in response to light, but usually only become light sensitive after imbibition. Germination of 'Grand Rapids' lettuce (*Lactuca sativa*), the weed species *Bidens pilosa*, some Australian daisies and many other small-seeded species is promoted by red light (R; 660 nm) but inhibited by subsequent exposure to far-red light (FR; 730 nm) — a classic photoreversible phytochrome response (Table 8.2 and see Section 8.4). Sunlight has a high R:FR ratio which signals to a seed that it is located in an unshaded position. However, chlorophyll in leaves filters out red light so that under a canopy there is relatively more far-red light; that is, a low R:FR ratio prevents germination where light is likely to be insufficient for most species. These seeds use light spectral composition as an indicator of likely total photosynthetic radiation. This is an example of secondary dormancy because it is induced only after seed dispersal (seed that is dormant when shed from the mother plant has primary dormancy). Seeds may lie dormant for months or years, germinating only when a tree falls in a forest or after a disturbance such as ploughing a field. In the latter case, phytochrome is being used mainly to sense light *quantity*. Deep burial in soil prevents germination of small seeds with inadequate resources to grow to the surface. In contrast, germination of *Spinifex hirsutus*, which grows on sand dunes, is inhibited by light. Dark conditions exist deeper in the dune where there is likely to be more moisture, nutrients and stable sand.

Many seeds will not germinate unless water content has been reduced by dry storage. This is a common adaptation in desert annuals, which experience a seasonal rhythm of water availability. In cereals such as barley and wheat, alternative treatments can be substituted (Table 8.3). Some seeds, for example *Ranunculus* and orchids, contain rudimentary embryos that must develop further before germination can occur. Symbiosis with a fungus supports embryo growth of many orchids, and inoculation is incorporated into *in vitro* propagation methods.

Stratification, or pre-chilling, the exposure of seeds to cool moist conditions, is in many ways similar to chilling of buds (see below). The optimum temperature is usually about 5°C for temperate species such as peach (*Prunus persica*) and apple (*Malus sylvestris*). Embryos removed from freshly harvested fruit

can germinate but growth is slow and abnormal. Normal growth is restored by chilling or exposure to long photoperiods, conditions which seeds in nature would eventually experience. In Australia and New Zealand, many alpine species require stratification. *Eucalyptus pauciflora* seeds collected from high altitudes respond to chilling but those of coastal populations do not, suggesting that natural selection has occurred, creating two ecotypes. For tropical species, chilling may operate at a higher temperature range, usually above 10°C.

Single or multiple dormancy mechanisms can ensure germination at an appropriate time, depending on the species (Table 8.3). Despite all the complex entrainment to environmental cues, many seeds will eventually germinate even without their normal signals, a failsafe mechanism ensuring some attempt at establishment before the seed's longevity expires.

Feature essay 8.1 Dormancy in wheat grains: nature and practical application

D. Mares

Ancestral wild wheats, the progenitors of modern bread and pasta wheats, were endemic to the eastern Mediterranean and possessed a number of mechanisms, including grain dormancy, which were requisite to their survival in that environment. Grain which ripened before the long, hot summer remained dormant, avoiding germination in response to chance rain, until the return of cooler, more rainy periods later in the year. Wheat is now cultivated worldwide in diverse environments, many of which have a high risk of rain and cooler weather during the harvest period. Unfortunately, during domestication and genetic improvement many of the mechanisms which reduced untimely germination have been inadvertently discarded or found to be incompatible with the requirements of large-scale commercial farming. Indeed, the relationship of grain dormancy to consistent grain quality has not always been taken advantage of by breeders.

In the absence of protective mechanisms, rain falling on ripe wheat crops may induce pre-harvest germination of grain (Figure 1), rendering it unsuitable for commercial processing. Sprouted grain in Australia has resulted in losses to growers of hundreds of millions of dollars. Breeders are therefore looking to reintroduce factors such as dormancy into new wheat cultivars to provide 'insurance' against pre-harvest rain. After first searching for dormancy characters in older cultivars held in the world wheat collections, the next hurdle is to transfer dormancy to elite cultivars which already possess all the other required agronomic, quality and disease-resistance characters.

There is a well-known association between red seed coat and dormancy, but white-grained genotypes with significant levels of dormancy have also been identified (Mares 1987). To date, the dormancy from red wheats has not been successfully transferred, in its entirety, into a white-grained background. Red-grained wheat cultivars dominate world production except in Australia where only white-grained genotypes are cultivated. Dormancy in both grain types is a transient character which develops during desiccation of the maturing grain, then decays with time after ripeness. Dormancy appears to be deepest if the grain has ripened in a cool environment but can be eroded by rain in the 20 day period leading up to harvest ripeness (Mares 1993). To rank genotypes for potential depth of dormancy, all lines need to be grown in the same environment and tested at the same stage of maturity using standard wetting treatments or germination tests.

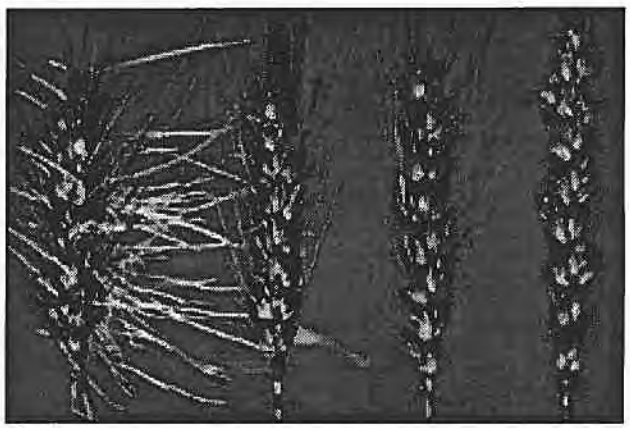


Figure 1 Lack of dormancy can lead to pre-harvest sprouting in wheat. Ripe spikes were subjected to a wetting treatment — an overhead spray for 2 h — then maintained at high humidity and 20°C for 5 d. The spike on the left is from a susceptible non-dormant cultivar which sprouted readily compared with three other more dormant, sprouting-resistant cultivars
(Photograph courtesy D. Mares)

Dormancy in wheat grains is dependent on the presence of an intact seed coat. Damage to this structure through invasion by fungal pathogens, disruption during swelling and shrinkage caused by wetting/drying cycles or through physical abrasion during threshing results in a loss of dormancy. Segregation patterns obtained in inheritance studies are consistent with control by two independent, recessive factors and indicate that dormancy is only recovered when both factors are present simultaneously. With simple Mendelian segregation, dormancy would have been expected in the F_2 generation. However, dormant segregants were not revealed until the F_3, one generation later than expected. From this, we can infer that at least one of the factors is probably expressed in the seed coat which lags one generation behind the embryo.

References

Mares, D.J. (1987). 'Preharvest sprouting tolerance in white grained wheat', in *Fourth International Symposium on Preharvest Sprouting in Cereals*, ed. D.J. Mares, 66–74, Westview Press: Boulder, Colorado.

Mares, D.J. (1993). 'Preharvest sprouting in wheat. 1. Influence of cultivar, rainfall and temperature during grain ripening', *Australian Journal of Agricultural Research*, **44**, 1249–1272.

8.1.3 Bud dormancy

Much of our knowledge of bud dormancy comes from temperate deciduous trees, especially fruit crops such as apples and stonefruit. Trees detect environmental signals, mainly shortening daylength and cold, which herald winter and trigger reductions in growth rate, onset of endodormancy, development of bud scales and leaf fall. As buds enter endodormancy, warm temperatures (>15°C) no longer promote growth. Several weeks or months of chilling (0–12°C) are required to overcome endodormancy. The plant then enters ecodormancy, when it will respond to warm temperatures with bud break. Note that break of endodormancy can therefore often occur weeks prior to growth resumption. In some tropical species such as coffee, water stress is an alternative cue for breaking flower bud endodormancy (Drinnan and Menzel 1994). Buds then exist in an ecodormant state ready to respond by rapid floral growth as soon as the first rains fall, heralding the end of the dry season (Figure 8.2).

Several models have been proposed to describe dormancy and to attempt to predict responses to different growing conditions. One problem is a lack of measurable indicators of endodormancy other than an inability to grow. Researchers typically quantify 'depth' of dormancy by the duration of chilling required to break dormancy, and then the ability of warm temperatures to 'force' bud growth on cut shoots, that is, after

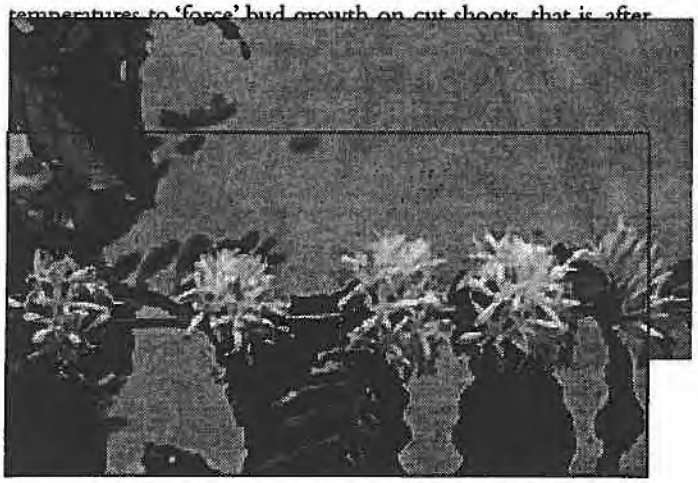


Figure 8.2 Synchronised anthesis of coffee (*Coffea arabica*), 10 d after restoring water supply to droughted trees. Endodormancy in coffee flower buds is broken by water stress, then buds remain in an ecodormant state until rain permits resumption of growth. This adaptation allows fruit development to coincide with periods of water availability. In cultivation, a drying-irrigation cycle can synchronise flowering which later leads to a shorter harvesting period
(Photograph courtesy C.G.N. Turnbull)

Entry into and exit from bud dormancy are often gradual transitions rather than abrupt events. Some researchers have represented these phases as sine wave oscillations, with measurable reference points (e.g. peak growth rate in summer and maximum dormancy in midwinter) which enable comparison of data from different sites (Fuchigami and Nee 1987).

Temperate crops in the tropics

Temperate fruit crops are increasingly being grown at lower latitudes (15–30°) than where they originate (30–50°). If endodormancy is still being overcome by chilling, then how little chilling is enough? A good model can allow estimation of whether a new location is suitable for production of particular fruit varieties prior to expensive orchard planting. For example, peach and nectarine varieties have been bred with low and high chilling requirements, suited to subtropical and temperate climates respectively. Early models resulted in rankings based on number of chill hours (usually below 7.2°C). Chilling required can vary from less than 50 h below 7.2°C for some subtropical 'low-chill' peach cultivars, up to 3000 h for some cultivars of pear (Table 8.4). A modified version, called the Utah model, equates a chill unit to 1 h at 6°C; higher and lower temperatures between 0–15.9°C have proportional positive effects, but temperatures above 16°C are inhibitory (Richardson *et al.* 1974). This temperate model is less accurate in warmer areas where the Erez *et al.* (1988) model, as modified by Batten and Firth (1987), often provides a more reliable estimate of date of budburst (Table 8.5). According to this model, effectiveness of chilling is enhanced by day temperatures of 15°C or less but negated by temperatures above 18°C. None of these models quantify the growth-permitting periods of warm temperature required for subsequent bud break, so an additional measure quantifies thermal units: the Growing Degree Hour where 1 h is allocated for each hour and degree above 4.5°C (Figure 8.3).

Table 8.4 Chilling requirements, in hours below 7°C, required to cause break of bud dormancy in some deciduous fruit crops

Fruit crop	Chill hours
Grape	None
Fig	Few
Almond	0–800
Kiwifruit	450–700
Peach and nectarine	50–1250
Apple and pear	200–3000
Cherry	800–1700

(Adapted from Saure 1985)

Table 8.5 Models based on accumulated 'chill units' give different predictions of time of bud break, which can be compared with actual dates of bud break. Data is for Sunred nectarine, a 'low-chill' cultivar grown at three subtropical locations in northern New South Wales (approx. 29°S). An refinement in the Batten and Firth version is inclusion of terms for temperatures >18°C, that is, above the normal chilling range, which are common in the subtropical winter and partially negate the chilling response. n/a = Completion of chilling not achieved, according to model

		Date of dormancy termination				
Location	Year	Actual	Predicted (Hours < 7.2°C)	Predicted (Utah)	Predicted (Batten & Firth)	Error (days) (Batten & Firth)
Bangalow	1982	30 Jun	19 Jun	20 Jun	20 Jun	+10
Bangalow	1983	23 Jul	14 Jun	27 Jul	19 Jul	+4
Bangalow	1984	4 Jul	7 Jul	n/a	9 Jul	−5
Tockombil	1984	3 Aug	n/a	11 Jul	29 Jul	+5
Tockombil	1985	14 Jul	1 Aug	28 Jul	18 Jul	−4
Rosebank	1985	18 Jul	16 Jul	26 Jul	23 Jul	−5

(Adapted from Batten and Firth 1987)

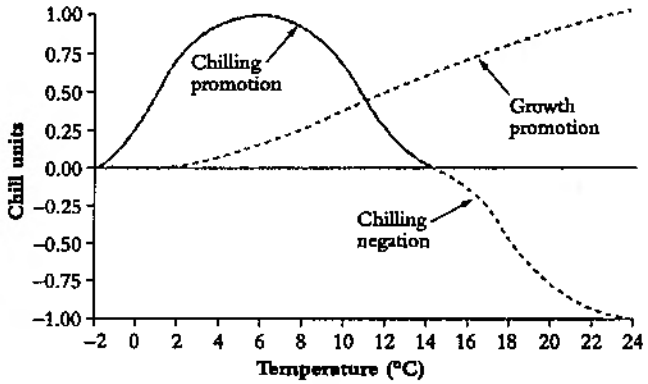


Figure 8.3 In many species, progress through bud dormancy then resumption of growth depends on temperature. Two factors are involved: first, the satisfaction of chilling requirements depends on suitable periods at low temperature (measured as chill units), but can be negated by temperatures above 15°C; second, temperatures above 4.5°C have a growth promoting effect, measured as thermal units
(Reproduced, with permission, from Seeley 1996)

What are the consequences of insufficient chilling, and are there alternative treatments? Symptoms of inadequate cold periods include delayed and weak leaf growth, delayed and protracted flowering, poor fruit development and irregular ripening. Potassium nitrate (KNO_3), thiourea and especially hydrogen cyanamide are simple chemicals that are effective substitutes for stimulating uniform budburst. The mechanisms by which these compounds work are not known, but growth regulators such as gibberellins, cytokinins and cytokinin analogues, in particular thiadiazuron, can also cause similar responses.

Apples are grown in the tropical and subtropical areas of Indonesia, peaches are grown in Venezuela and table grapes are grown in Thailand, Venezuela and southern India where no chilling occurs (Subhadrabandhu and Chapman 1990). Growth of buds is stimulated by chemical (sodium chlorate, copper sulphate or urea) or manual defoliation or pruning immediately after harvest thus breaking endodormancy before it enters its 'deep' mid-winter phase. Cyanamide treatment has enabled out of season production of table grapes in tropical Queensland. Irrigation then promotes uniform budburst and cropping under otherwise dry conditions. At least two harvests are possible each year and cycles can be staggered, giving almost continuous fruit supply.

8.1.4 Physiological control of dormancy

(a) Hormones as regulators?

Currently we know more about the environmental factors that influence dormancy than about the physiological mechanisms of dormancy. Here we attempt to draw together common features of the diverse types of dormancy in buds and seeds, in particular examining whether inability to grow relates to hormonal factors (Dennis 1994).

Links between genome and physiological processes are illustrated by single-gene seed dormancy mutants, which are either abscisic acid (ABA)-deficient (weak dormancy) or gibberellin-deficient (extra-deep dormancy) (Karssen and Groot 1987). Induction of seed dormancy is clearly linked to ABA, and gibberellins are required for germination, so in a gross sense these hormones need to be present for normal processes to proceed. Applied hormone experiments lead to similar conclusions: although ABA does not usually prevent break of dormancy, it can inhibit germination and bud growth, often opposing the effects of gibberellins, cytokinins or ethylene. Seeds with various dormancy mechanisms may respond to one or more plant growth regulator (Table 8.3), but there are many reports of germination failure or abnormal seedlings. Light requirement of lettuce and dry storage requirements of barley are overcome by applied gibberellins, but antagonised by applied ABA. Likewise, budburst in peach and apple is promoted by a mix of gibberellin and cytokinin, but inhibited by applied ABA. Cytokinins promote some germination in lettuce but are less effective than gibberellins in most species. Ethylene stimulates germination in celery (*Apium graveolens*), peanut (*Arachis hypogea*) and cocklebur (*Xanthium strumarium*). One conclusion is that a complex balance of inhibitors and promoters regulates entry to and exit from dormancy. Put another way, there are at least two control points and meristem growth may be prevented by either high concentrations of inhibitors or insufficient promoters.

However, data on endogenous plant hormone concentrations do not always support the notion of control by changes in levels of active substances. Quantities of applied

plant growth regulators required to cause a response usually vastly exceed normal endogenous content, for example the amount of applied gibberellin required to stimulate barley germination. Rightly, this has led to re-examination of the control mechanisms. Trewavas (1982) argued that tissue 'sensitivity' to hormones, that is, capacity to respond, changes with development and environmental stimuli, and that this sensitivity is a major controlling factor. Indeed, phases of sensitivity and insensitivity to applied gibberellins and ABA appear to operate during development, dehydration and dry storage of sunflower seed (Figure 8.4). Other supporting evidence comes from gibberellin- and ABA-insensitive mutants which fail to respond to these hormones regardless of endogenous or applied concentration. Alterations in hormone levels due to

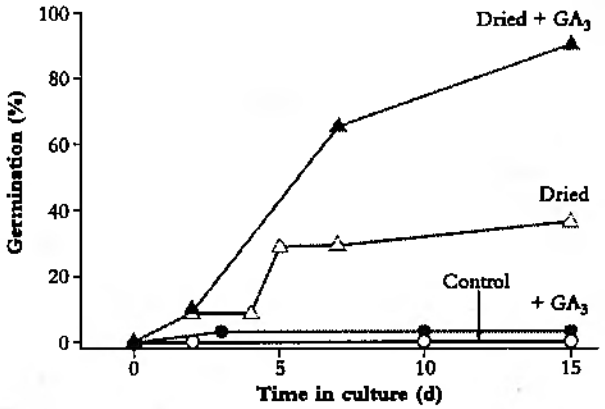


Figure 8.4 Responsiveness of sunflower embryos to applied gibberellin (GA) is seen only when dormancy has been partially released. Embryos were cultured on 5 μM gibberellic acid (solid symbols) or control medium (open symbols), before (circles) or after (triangles) a 3 d drying treatment which partially broke endodormancy
(Redrawn, with permission, from Le Page-Degivry *et al.* 1996)

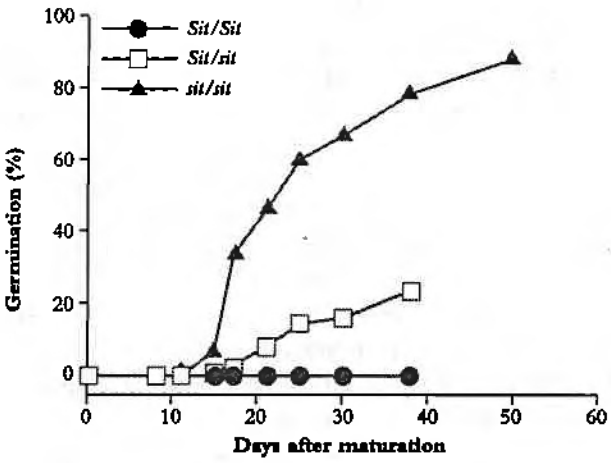


Figure 8.5 Vivipary in wild-type tomato (*Sit/Sit*, i.e. ABA-synthesising) and ABA-deficient tomato (*sit/sit*). No seeds germinated within ripe tomato fruits derived from self-pollinated *Sit/Sit* plants. Juice of ripe *Sit/Sit* fruits contains 0.84 μmM ABA and each seeds contains 7 pmol ABA. In contrast, vivipary occurred in most *sit/sit* tomato fruits which only have only 0.08 μmM ABA and 0.8 pmol ABA per seed. Self-pollinated *Sit/sit* plants would contain seed of both gphenotypes but the mother plants would have possess the dominant *Sit*, allowing ABA synthesis. A quarter of the seed (those carrying *sit/sit*) would be viviparous but three-quarters (those carrying *Sit/Sit* and *Sit/sit*) would not be
(Based on Groot and Karssen 1992)

mutation are generally much more severe than changes that occur in wild type plants as a consequence of environmental factors. ABA-deficient tomato (Figure 8.5) and *Arabidopsis* mutants fail to enter normal dormancy because of a lack of increase in embryo ABA. Surrounding seed tissues absorb most applied ABA without translocating it to the embryo, which may also explain failure of seed dormancy induction with applied ABA.

So what is the role of ABA in induction of seed dormancy? In late embryogenesis, ABA concentration increases as water potential decreases. Elsewhere in the plant, responses to altered water potential are also mediated by ABA, typically those associated with water stress (see Section 9.3). ABA alters transcription of a suite of genes, resulting in cessation of synthesis of reserve and other proteins, and modified transcription of some *Lea* genes (late embryogenesis abundant; see Chapter 10). In cotton, one class of *Lea* mRNAs increases coincidentally with ABA but another class responds only to drying. *Lea* genes code for a class of proteins found in many species including cotton, pea and cereals. These proteins are strongly hydrophilic, highly stable and are able to maintain a locally water rich environment at the subcellular level. This may be critical in desiccation tolerance associated with the dormant state.

There is a tenuous association of endogenous inhibitors with release (as distinct from induction) of bud or seed dormancy. Early research suggested a close correlation of progress of dormancy with inhibitors including phenolics such as naringenin in peach and phloridzin in apple, and ABA in several fruit crops. However, endogenous ABA declines in chilled apple buds which burst to produce new shoots, but also in buds never exposed to chilling temperatures which remain dormant. In both chilled and non-chilled apple seeds, ABA levels do not change more than two-fold but only chilled seeds germinate (Figure 8.6). ABA content is similar in dormant and non-dormant wheat but ABA-responsive genes are more abundantly expressed in dormant wheat seeds, implying existence of alternative regulatory factors and perhaps non-transcriptional control of the relevant genes. Embryo endodormancy may therefore be maintained by ABA in only a few species, such as sunflower (*Helianthus annuus*), where treatment of dormant excised embryos with fluridone, an inhibitor of ABA synthesis, results in growth.

Can we instead assign control of dormancy break to promotive compounds? Gibberellins are probably the best candidates, based on widespread responses to applications of this class of hormone. In *Salix pentandra*, where short days induce dormancy and long days release it, a transient increase in active shoot gibberellin (GA) content is detectable within one day of transferring from short days to long days (Figure 8.7). In hazelnut, endogenous gibberellins are not modified by chilling but GA_1 content rises 40-fold *after* transfer to warm conditions suitable for germination, suggesting a role in growth promotion as distinct from dormancy release. Likewise, in wild oats (*Avena fatua*), 'after ripening' dry storage

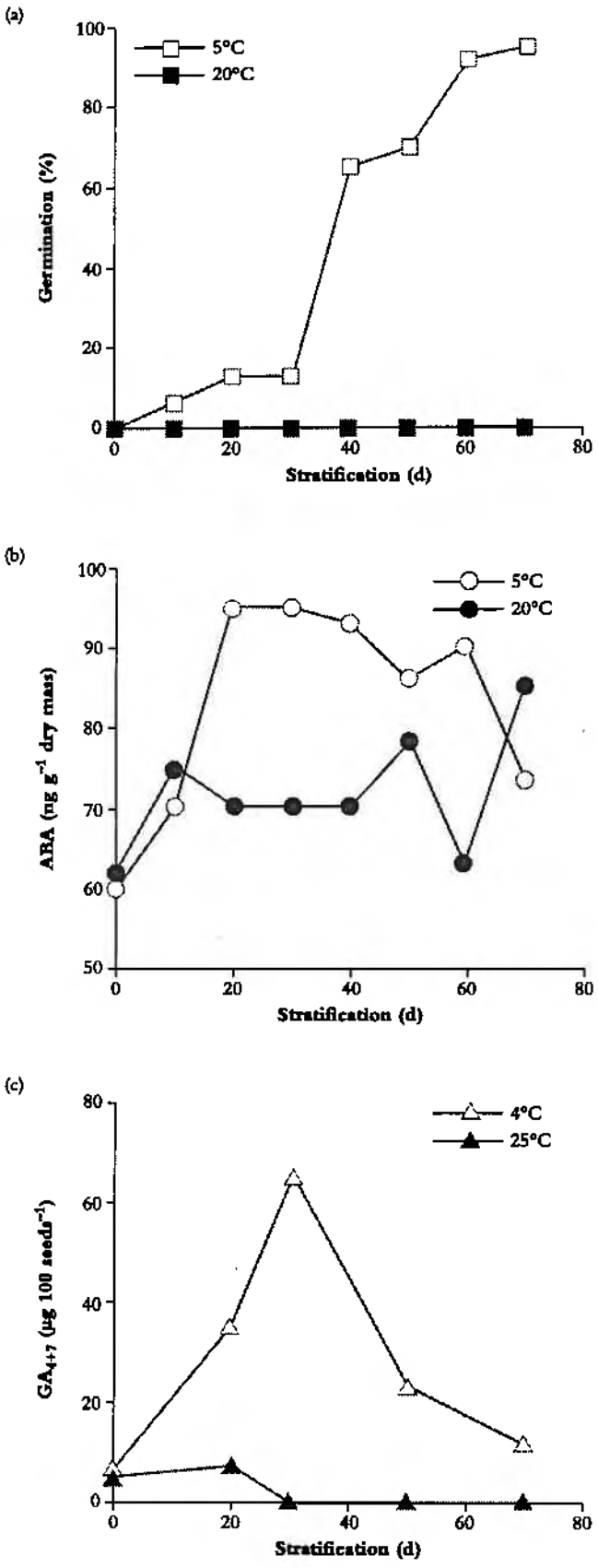


Figure 8.6 Endogenous gibberellin and ABA levels during breaking of dormancy in apple seeds exposed to cold (4–5°C) or warm (20–25°C) temperatures. (a) Germination is dependent on cold treatment. (b) Embryo abscisic acid levels do not decline during cold treatment or during germination. (c) Seed gibberellin (GA_{4+7}) levels increase transiently as seed start to germinate (Based on Subbaiah and Powell 1992 and Halinska and Lewak 1987)

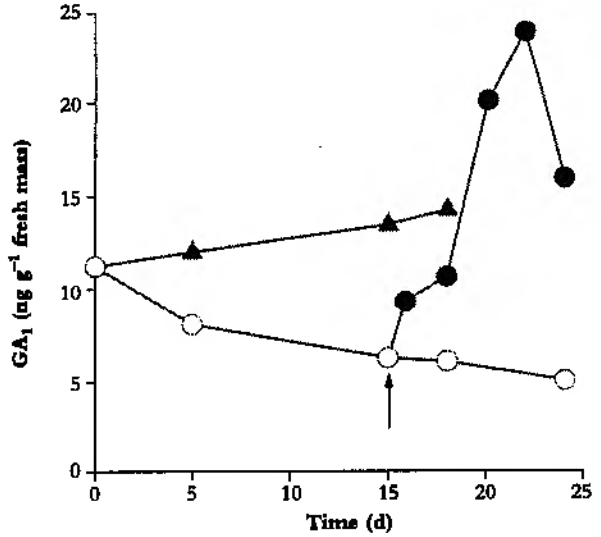


Figure 8.7 Bud dormancy in *Salix pentandra* is broken by long days, and results in a transient increase in active gibberellin (GA_1) content of shoot tissue within one day of transfer from short days to long days (●). Arrow indicates day of transfer. Plants in continuous long days (▲) or short days (○) show only slow changes in gibberellin levels
(Redrawn, with permission, from Olsen et al. 1997)

releases seed dormancy but has no effect on endogenous gibberellin levels until imbibition, when gibberellin biosynthesis is substantially enhanced. Light requirements can often be replaced by applied gibberellins, and gibberellin-biosynthesis inhibitors can prevent light-stimulated germination. Endogenous gibberellins increase with chilling and dry storage in *Arabidopsis*, and with light exposure in lettuce. Gibberellin-deficient *Arabidopsis* mutants do not germinate unless gibberellin is supplied, and this response is independent of ABA content. However, changes in endogenous gibberellins in wild-type *Arabidopsis* are less conclusive, suggesting that altered gibberellin sensitivity may contribute to normal germination control. We are just beginning to understand tissue sensitivity and hormone signal transduction pathways (Section 9.3.1). To conclude, there are some species where there is good evidence for ABA-induced dormancy and gibberellin promotion of meristematic activity but these are not necessarily universal mechanisms. Hormone turnover, conjugation, compartmentation, receptors and signal transduction systems all represent potential control points, and all merit greater attention.

(b) Alternative indicators of dormancy

The hormonal models described above have limitations and some researchers contend that they represent oversimplifications of a complex set of interactive cyclic processes including organogenesis, internode elongation and bud leaf expansion (Crabbe 1994). Biochemical markers such as nucleic acid metabolism and membrane permeability, rather than morphological or physiological characteristics, can also indicate relative depth of dormancy between tissues and organs, and between meristems and submeristems. Adenylic nucleotides are required to maintain basal metabolic activity and even dormant tissues supplied with adenosine increase

their adenylic nucleotide (ATP) content. During dormancy break in buds of *Helianthus tuberosus* (Jerusalem artichoke) tubers, levels of both adenylic and non-adenylic nucleotides (NATPs = sum of guanylic (GTP), cytidylic (CTP) and uridylic (UTP) nucleotides) rise as tissues convert ATPs to NATPs, which are essential to sustain growth (Gendraud 1977).

In stems, trunks and developing tubers bearing dormant buds, storage parenchyma acts as a strong sink during metabolite accumulation while nutrient movement into bud meristems may be impeded. Breaking dormancy appears to remove this block and is part of the changes that permit resumption of growth. Water status also influences dormancy. Dormant seeds and sometimes buds have lowered water content which limits metabolism and often assists survival (Vertucci 1989; Faust *et al.* 1995). Metabolic activities for growth require free water (bulk cellular water) but cannot occur in the bound water associated with macromolecular surfaces. Water content therefore determines the possible types of reactions: at low seed water content (0–8%) only catabolic and non-enzymatic activity occurs, but >25% water content is required for integrated processes such as mitochondrial electron transport and protein synthesis. Water content also determines the ability of seeds to perceive and respond to environmental cues. Apple seeds become sensitive to chilling temperatures only if hydrated to >8% water content, and many seeds such as the weedy coloniser species *Bidens pilosa* acquire light sensitivity only after imbibition.

Water content in bud tissue is generally higher and varies less but may still have a regulatory function. The state of water has been visualised in vegetative buds by using nuclear magnetic resonance imaging. Free and bound water content correlate strongly with bud dormancy release and chilling in low- and high-chill cultivars of apple, Anna (400–700 chill units, typical of subtropical regions) and Northern Spy (2600–3600 chill units, typical of the temperate zone). Very little free water (about 30%) is detectable in bud meristems at the beginning of endodormancy, but this increases to 70–80% after 400 h at 4°C in Anna and 3000 h in Northern Spy. Seed germination also requires free water, with metabolic activity suppressed in seeds having a water content below 30%. High osmotic potential of tomato fruit tissues may be partly responsible for seed dormancy by keeping seed water content low during late stages of development. With the exception of hard-coated species, most dormant seeds hydrate easily but this does not necessarily lead to immediate germination.

(c) Conclusion

Dormancy remains an intriguing but complex phenomenon. Clearly, plants are well attuned to making use of environmental cues. The ability to enter a period of latent life is remarkable in itself, all the more because plants in effect anticipate adverse conditions before their onset, and thus dormancy can be established in advance. However, there is no single hypothesis to account for induction, maintenance and breaking of dormancy which is consistent across all species. Interactions of many metabolic and cellular processes with many genes are probably linked to hormonal signals. We need to appreciate more that hormonal control is intrinsically complex, and directly and indirectly influences genome expression, while mediating some environmental cues. Dormancy is a prime example of genotype × environment interaction. Plants use external signals to time entry into a 'shutdown mode' (endodormancy, paradormancy), then transition to a 'standby mode' (ecodormancy), but have internal controls to prevent inappropriate exit, instead foreshadowing future favourable conditions. Continuing studies with single-gene mutants and transgenic plants (Chapter 10) should unlock some of dormancy's secrets.

8.2 Plant and organ orientation

Vascular plants orient themselves in space to optimise shoot exposure to radiant energy and CO_2 in the atmosphere, and to maximise root access to water and nutrients in the soil. To achieve this, there is a range of directional control systems, which change as a plant proceeds through its life cycle. Regardless of how a seed falls to the ground, on germination a seedling root grows downwards and the shoot grows upwards. What controls these opposite directions of growth?

First, seedling shoots are very sensitive to low-intensity *light*, curving strongly towards any directional light which may indicate a break in the leaf canopy that the shoot can utilise. In mature plants, leaf orientation can follow the sun during the day to maximise light capture, but if mid-day radiant energy becomes excessive the leaf blade may instead orient at right angles to the sun's rays. Flower buds are usually bent downwards, but on opening the stem straightens and holds the flower upright to maximise exposure to insects and other pollinating agents.

Second, *gravity* is an all-pervasive and constant orienting signal. However, roots and shoots generally show opposite responses to gravity, reflecting the intrinsic polarity in all higher plants. One half, the root system, is adapted for life in dense dark soil, while the other half, the shoot system, has evolved to exist in the fragile atmosphere, and harvests sunlight for photosynthesis. Conforming with this dichotomy, main roots exhibit a positive directional response to gravity, whereas shoots generally show a negative reaction.

8.2.1 Tropisms

Directional growth responses to directional stimuli are called tropisms. There are three main kinds:

1. Gravitropism — gravity sensing
2. Thigmotropism — touch sensing
3. Phototropism — light sensing

The characteristics of the major tropisms are shown in Table 8.6. All these responses are due to different growth rates on two sides of a responding organ, resulting in curvature either towards or away from the stimulus. The positioning, or orientation in space, of many plant organs can be due to several tropisms and nastic (non-directional) responses acting together.

Table 8.6 Characteristics of four types of plant tropism. Positive means growing towards a directional stimulus, and negative means growing away. Plagiotropism is growth at an angle to gravity

Tropism	Stimulus	Response	Examples
Gravitropism	Gravity	Positive	Primary roots
		Negative	Shoots
			Leaves
		Plagiotropism	Axillary branches
			Lateral roots
			Some leaves
			Runners
			Rhizomes
Phototropism	Light	Positive	Shoots
			Leaves
			Coleoptiles
		Negative	Some types of shoot
			Some types of root
			Tendrils
Heliotropism	Light	Following the sun	Leaves
Thigmotropism	Touch	Positive	Tendrils of climbing plants
			Stems of vines

8.2.2 Gravitropism

As the primary root emerges from a germinating seed, it shows strong positive gravitropism leading to rapid downward curvature (Figure 8.8a). This enables the root tip quickly to penetrate the soil, giving anchorage and access to water, the latter being a vital factor in successful establishment. Root gravitropism has been investigated for over a century, but its mechanism is still not fully understood. However, we do know that gravity is detected in the root cap, and that normally both root cap and root tip need to be present for straight growth and curvature to occur. Because the elongation zone is situated behind the tip, information about the root's position must be transferred from the sensing site in the cap to the elongation zone.

Shoots sense gravity differently. Both the shoot tip and the growing zone behind it can detect and respond to gravity (Figure 8.8b), so that even decapitated shoots retain an ability to curve upwards when displaced from the vertical. The shoot tip, unlike the root tip, is therefore not essential for gravitropism.

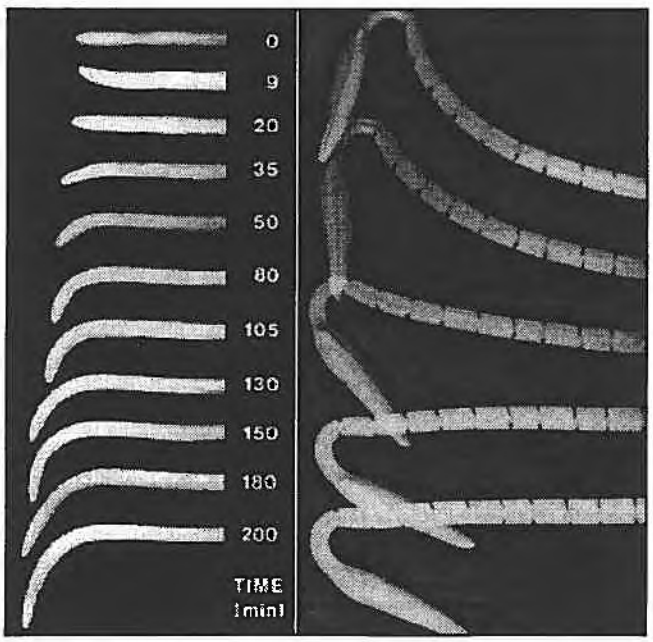


Figure 8.8 Time-lapse photographs showing gravitropism responses in horizontally placed roots and shoots. (a) Negative shoot gravitropism of a dark-grown cucumber seedling photographed at 15 min intervals. The ink marks on the hypocotyl are 2 mm apart. Upward curvature commences by 30 min due to simultaneous initiation of differential growth along the whole hypocotyl. (b) Positive gravitropism in a maize root. The initial slightly upward curvature is not unusual. Downward curvature commences around 30 min and continues as the tip grows forwards. By 150 min, the root tip has been restored almost to vertical
(a) From Cosgrove 1990; (b) from Pickard 1987)

8.2.3 Gravity perception

Detecting the direction of gravity is the essential first step in gravitropism. Plant organs achieve this by sensing the movement and position of starch grains contained within amyloplasts of specialised cells called statocytes (Figure 8.9a).

(a) Roots

In roots, statocytes are located in the root cap (Figure 8.9b) which also serves to protect the root meristem from abrasion by soil particles as it grows through the soil. Root cap involvement was first demonstrated in maize, when a needle was used to prise off the root cap. This procedure did not inhibit growth, but ability to sense and respond to gravity were completely lost until a new cap grew over the root tip about one day later. Subsequently, a gravity-insensitive mutant of maize was found that does not secrete the mucilage which normally covers and protects the root cap and tip. Mucilage artificially applied to mutant roots immediately restored the gravity response indicating that the root cap transmits information through the mucilage. This information is probably in the form of a small diffusible molecule, moving either in the mucilage or through the root apoplasm. Researchers have not yet been able to identify this chemical.

(b) Shoots

In dicotyledonous shoots, statocytes form a cylindrical tube one cell thick, which surrounds the vascular tissue (Figure 8.9c).

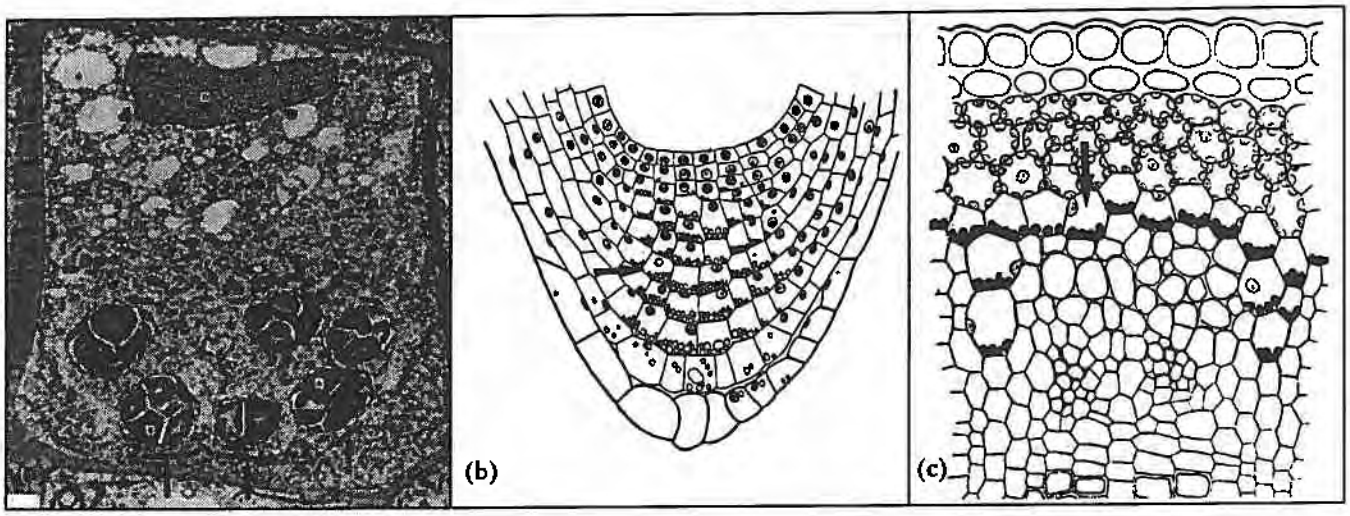


Figure 8.9 Sites of gravity perception. (a) Transmission electron micrograph of a statocyte cell in a root showing six statoliths (amyloplasts; a) each with a boundary membrane and containing two to four starch grains. Characteristically, the statoliths are resting on a network of endoplasmic reticulum (arrowed), which may be able to sense their movement. n, nucleus. (b) Longitudinal section through a root cap showing statocyte cells (arrowed) near the centre. (c) Transverse section of a primary stem showing layer of starch-containing cells (arrowed) which make up the starch sheath ((a) Reproduced, with permission, from Sievers and Volkmann 1977; (b), (c) reproduced from Haberlandt 1914)

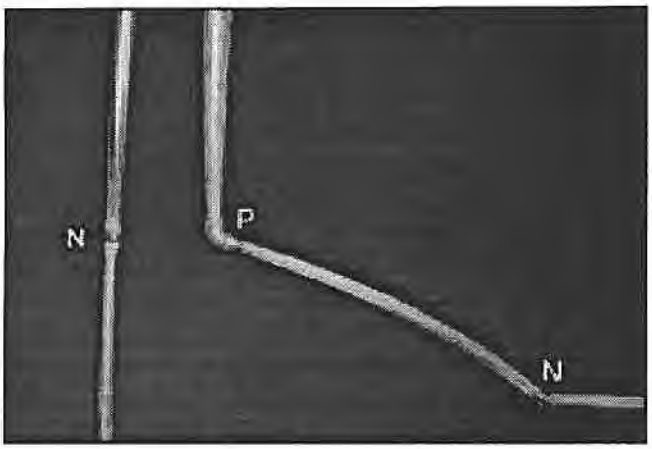


Figure 8.10 Gravitropism in a grass stem, due to combined responses of stem nodes (N) and basal leaf pulvinus (P). The stem on the right was placed horizontally one week before the photograph was taken and it now shows 30° upward curvature in the stem node right and 60° upward curvature in the leaf pulvinus, restoring the end of the stem to the vertical position (Photograph courtesy J.H. Palmer)

This cylinder is known as the 'starch sheath', because numerous starch grains show up very clearly in stem sections stained with starch-specific iodine solution. These statocytes are distributed along the length of the shoot and so can sense gravity in the absence of the apex. In grasses and cereals, stem statocytes are restricted to the stem node and leaf sheath pulvinus. Consequently, only the nodes and pulvini respond to gravity (Figure 8.10).

(c) Statocyte operation

The involvement of statocyte starch grains in gravity perception was proved by keeping barley plants in the dark for 5 d, which resulted in disappearance of starch grains as the starch was consumed in respiration. These starchless plants completely lost their gravity response, but feeding with sucrose resulted in stareh grains reforming and restoration of gravity sensing. Additional evidence comes from a maize mutant known as *amylomaize*, which has abnormally small starch grains and very slow gravitropic response.

Proof that the controlling force is gravity, and not, for example, lines of magnetic field, comes from experiments in which a centrifugal force was substituted for gravity. If a germinating bean seed was placed at the axis of a horizontal centrifuge rotating at one revolution per second, to give an acceleration of $4 \times 10^{-3} g$, this effectively counteracted gravity. The starch grains in the root cap developed in the centre of the cell and were unable to generate a displacement message. Consequently, the root remained straight. At two revolutions per second, equivalent to $2 \times 10^{-2} g$, the starch grains were forced against the outside wall of the statocytes. As a result, the root commenced to curve, bringing the tip parallel with the centrifugal force, that is, growing radially outwards. Now the centrifugal force acted along the length of the root and the starch grains were displaced onto the normally lower sides of the statocyte cells in the root cap, leading to straight growth. Experiments on plants under 'micro-gravity' conditions in space orbit have confirmed much of what was previously deduced from experiments on earth (Halstead and Dutcher 1987).

How do amyloplasts enable gravity sensing? Because of their high density and relatively large mass, they normally occupy the lowest part of the statocyte. When a root is displaced from the vertical, statocyte orientation is changed and the starch grains roll or slide 'downhill' through the cytoplasm to reach the new low point. Statocytes, possibly through stretch or displacement receptors in the plasma membrane, are able to recognise that starch grains have moved to new positions. An asymmetric message is then transmitted from the root cap to the growing region and a correction curvature is initiated until the cap returns to vertical. Similar events occur in shoots.

(d) Plagiotropism

Many organs naturally grow at an angle to gravity. This is a type of gravitropism termed plagiotropism and occurs in lateral shoots and roots, and also in some prostrate primary shoots, for example runners of strawberry and subterranean rhizomes of some grasses and sedges (Figure 7.18). The lateral growth angle is variable but is at least partly under genetic control, giving every plant a recognisable architecture. In shoots, the angle is also influenced by the vertical primary stem and by environmental factors. For example, exposure to bright sunlight tends to increase the angle to the vertical, while shade reduces it. The runners of couch grass illustrate the requirement for exposure to direct sunlight. When their shoots grow into shade, the plagiotropic tendency disappears and stems grow vertically in search of higher light intensity. The primary shoot apex also influences direction of growth of lateral shoots, which often changes to vertical if the primary shoot tip is removed. This response is probably linked to apical dominance.

8.2.4 Thigmotropism

Tendrils are specialised thread-like structures that can grasp objects with which they come into contact. They are modified leaves or stems sensitive to sliding and/or repeated touch, such as occurs when a tendril contacts a neighbouring stem. Tendrils enable climbers and vines which have slender non-self-supporting stems to access sunlight at the top of the vegetation cover with less investment in shoot biomass per unit height gain. In effect, tendrils search for surrounding objects because the end of the tendril makes wide spontaneous sweeping movements as it grows. On contact, the touch stimulus induces the tendril to coil around the object as a result of the cells on the non-stimulated side expanding more rapidly than those on the side making contact (Figure 8.11). Coiling is a tropic response, since direction of curvature relates to the direction of touch. Touch stimulation is continued during coiling so that tendrils ultimately twine several times around the object. The rest of the tendril may then show spontaneous coiling which effectively pulls the stem nearer to the contacted object, giving mechanically superior support (Figure 8.12). This second phase is often in the opposite helical direction and may be initiated by tension.

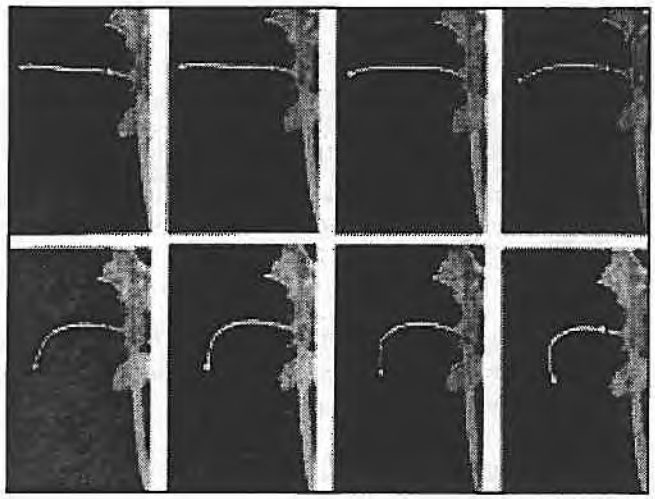


Figure 8.11 Initial thigmotropic curvature after touch stimulation can be very rapid. Time-lapse photographs, at 10 s intervals, of watermelon tendril following 10 s of touch stimulation. Compare the time-scale here with much slower responses in Figure 8.8.
(Reproduced, with permission, from Carrington and Esnard 1989)

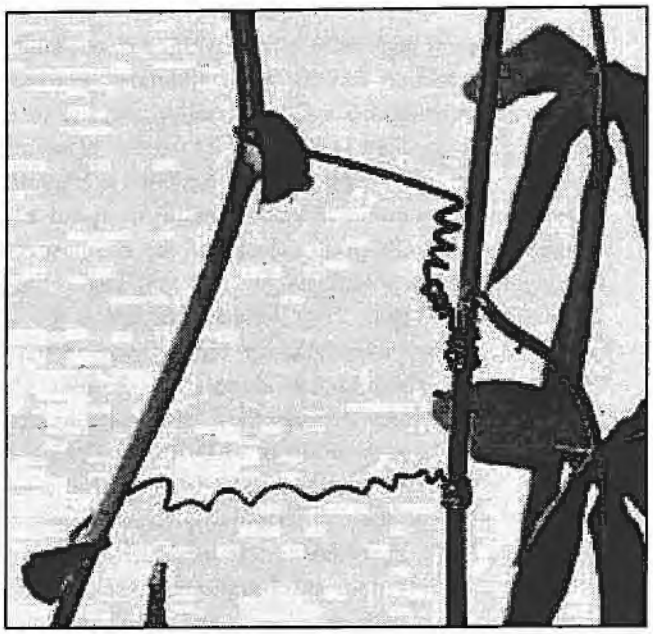


Figure 8.12 Thigmotropic twining of a tendril around a supporting stem, after touch contact by one side of the tendril. Later, tension coiling within the tendril has dragged the stem towards the support
(Photograph courtesy J.H. Palmer)

Tendrils detect contact via sensory epidermal cells called tactile blebs. These cells are rich in microtubules and actin filaments, suggesting an involvement of the cytoskeleton. Touch sensing by the sensory bleb is converted to a sigual which results in coiling commencing only a few seconds after contact. Coiling is due partly to changes in cell turgor and partly to differential growth along opposite sides of the tendril.

8.2.5 Phototropism

Phototropism is a curvature in relation to directional light. In ferns, conifers and flowering plants, positive phototropism, that is, curvature towards the light source, is the dominant response. Phototropism assists cotyledons and emerging leaves to maximise light interception for photosynthesis, before a seedling's food reserves are exhausted. Seedlings of some tropical vines, for example *Monstera* and *Philodendron*, are instead negatively phototropic and direct their stems towards the shadow cast by tree trunks, which these vines need for support. Among lower plants, filamentous algae can grow towards or away from a light source and in bryophytes sporophyte stalks show positive phototropism.

Phototropism appears to occur in three stages: light perception, transduction and curvature. Illuminating a seedling from one side establishes a light gradient across the width of the stem, because light is absorbed by various pigments. By measuring the positive phototropic response to exposure to different wavelengths of light, an 'action' spectrum can be established (Figure 8.35). In coleoptiles, this action spectrum has major peaks in the ultraviolet (370 nm) and in the blue region (420–475 nm). This stimulated a search for chromophores which efficiently absorb blue light and resulted in carotenoids and flavins being identified as possible phototropic sensors. Rapid progress in the 1990s has led to identification of a flavin, in the form of FAD (flavin adenine dinucleotide), as the chromophore which is coupled to a soluble protein to generate the complete flavoprotein photoreceptor (Cashmore 1997). Potassium iodide inhibits light absorption by flavins and can reduce phototropic responses. During the transduction stage in etiolated grass and cereal seedlings, the absorbed blue light may cause auxin (indoleacetic acid, IAA), which normally moves down the shoot from the tip, to migrate towards the shaded side. This would promote more elongation in the shaded side than in the illuminated side, causing bending towards the light during the subsequent growth response. Evidence for redistribution of IAA, rather than its destruction on the illuminated side, comes from experiments in which stem segments were placed vertically on agar receiver blocks after the stem tip had been cut off to remove the source of naturally produced IAA. An agar block containing ^{14}C IAA was then placed on the apical end of the stem segment. When the stem segments were illuminated on one side, it was found that distribution of ^{14}C label in agar receptor blocks on the illuminated and shaded side was in the ratio of 25:75, and in the tissue was 35:65 for the illuminated and shaded halves. Of course, the label may have been converted to other compounds and endogenous auxin in intact plants may behave differently. Indeed, no IAA gradient is found in many graviresponding tissues (Mertens and Weiler 1981). We must therefore conclude that *gross* IAA redistribution is not the only cause of phototropic bending. An alternative explanation is that IAA may need only to move between adjacent tissue layers, perhaps from the cortex to the more-auxin-sensitive epidermal cells (Macdonald and Hart 1987). Because unilateral illumination does induce other rapid changes in stem cells, leading to growth inhibition on the illuminated side and curvature towards the light source, there may be no need to invoke a long-distance signal such as auxin.

Heliotropism is a variation of phototropism where the leaf lamina and apical bud respond to changes in direction of the sun's rays, and track the movement of the sun. Generally, inclination to the sun remains constant during the day and this optimises radiation interception. Sunflower leaves and flower heads provide a good example (Figure 8.13). In leaves, lamina inclination in the daytime is controlled by diurnal petiole straightening, curvature and rotation. During the night, leaves

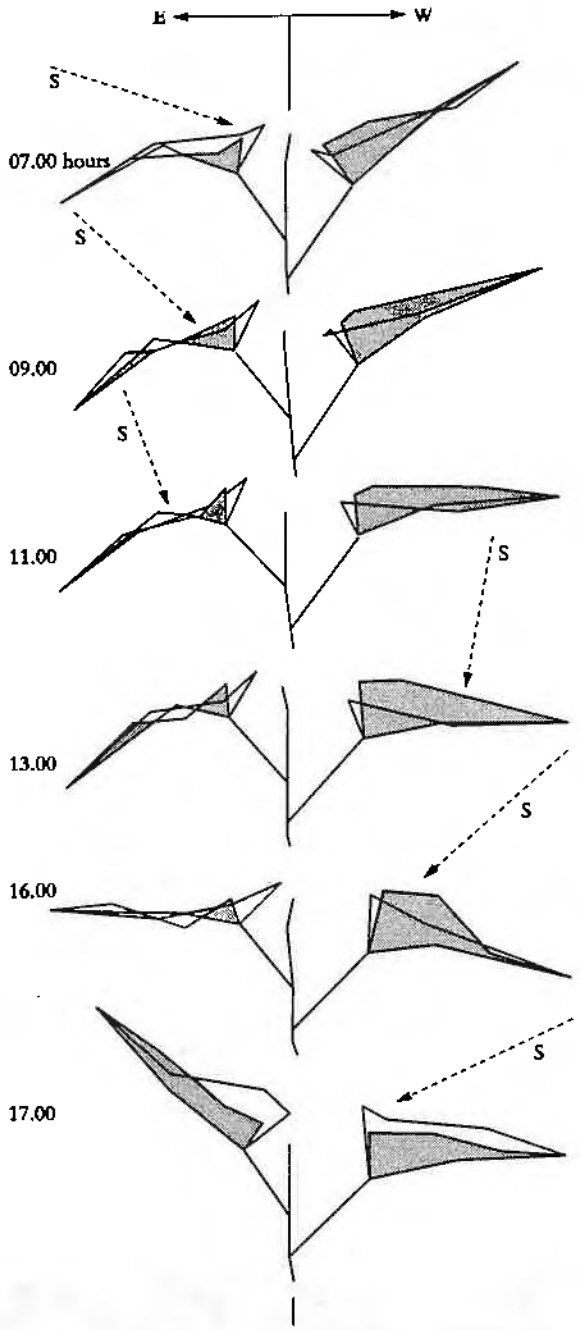


Figure 8.13 Diagrams of heliotropic movement of sunflower leaves from 7 am to 5 pm. Lamina inclination changes for leaves on the east (E) and west (W) sides of the plant, so that they maintain a relatively constant angle to the solar beam (S), as the sun moves from east to west during the day. During the night, leaf positions recover to their starting point. Lamina inclination is controlled by curvature of the petiole, which is not shown in these drawings (Reproduced, with permission, from Lang and Begg 1979)

return from a westerly inclination at sunset to face east at sunrise. Heliotropic leaf movement is dependent on continued petiole growth and ceases at leaf maturity.

Overall models for control of tropisms

The pioneering studies on auxin responses in coleoptiles have undoubtedly influenced present-day models, yet vigorous debate among researchers continues on the wider importance or otherwise of auxin in tropisms, especially where sensing

Table 8.7 Types of growth differential induced during tropic responses. All these tropisms result in redirection of the growing tip, but how this is achieved varies. The only option not represented here is differential acceleration of both sides, presumably because an overall increase in growth rate is more difficult to sustain in tissues that were already growing before the response started

Tropism type	Species	Organ	Nature of growth differential		
			Faster side	Slower side	Source
Phototropism	Oat	Coleoptile	0	–	Franssen *et al.* (1982)
	Cress	Hypocotyl	0	–	Franssen *et al.* (1982)
	Cucumber	Hypocotyl	0	–	Franssen *et al.* (1982)
	Mustard	Hypocotyl	+	–	Rich *et al.* (1987)
Gravitropism (–)	Sunflower	Hypocoryl	+/0	–	Carrington and Firn (1985) Berg *et al.* (1986)
	Cucumber	Hypocoryl	+	–	Cosgrove (1990)
	Wheat	Node	+	0	Bridges and Wilkins (1973)
Gravitropism (+)	Pea	Root	+	–	Konings (1995)
	Cress	Root	+	–	Selker and Sievers (1987)
	Maize	Root	+	0	Barlow and Rathfelder (1985)
			–	–	Evans *et al.* 1986
	Wheat	Root	–	–	Rufelt (1971)

+= stimulation of growth compared with previous rate;
0 = no change compared with previous growth rate;
– = reduction of growth compared with previous rate;
— = reduction greater than that observed on other side of organ.
Information derived from Firn and Digby 1980 and Hart 1990

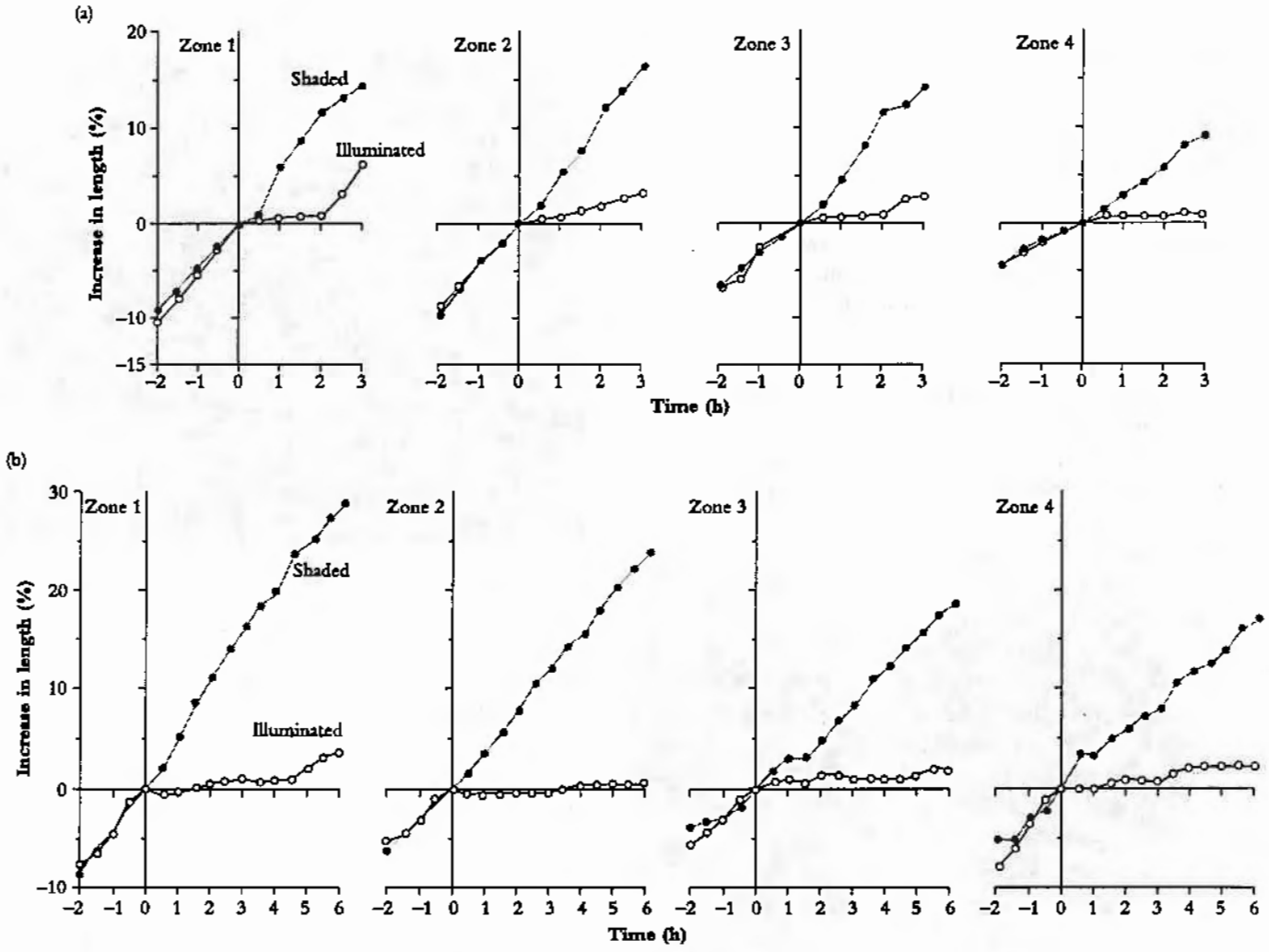


Figure 8.14 Differential growth during phototropic response of oat (*Avena sativa*) coleoptiles. Curvature is due to growth in all zones of the coleoptile stopping simultaneously on the illuminated side, but continuing unchanged on the shaded side. Zone 1 is nearest the apex. (a) Intact coleoptiles; (b) the response remains the same in intact coleoptiles with tip covered by a black cap, rotating on a horizontal clinostat at 1.2 rpm.
(Reproduced, with permission, from Franssen *et al.* 1982)

and responding cells are the same (Trewavas *et al.* 1992). Some researchers have attempted to generate a single model to explain all the types of differential growth that are represented by tropisms. Early researchers, including Charles Darwin, measured responses by angle of curvature either towards or away from the stimulus. However, detailed kinetic analysis has revealed that, perhaps surprisingly, there are at least four versions of growth differential. Some involve growth acceleration and some, deceleration (Table 8.7; Firn and Digby 1980). It is hard to envisage a single growth-regulating chemical, whether auxin or not, being laterally redistributed and causing sometimes net growth promotion, sometimes net growth inhibition and sometimes no change at all in growth rate on one side of the organ (Franssen *et al.* 1982). Coleoptile tips are very sensitive to light and may initiate a basipetal wave of growth-regulating chemical, but it is difficult to reconcile this notion with the observations that (a) all growing regions of oat coleoptiles initiate a response at the same time (Figure 8.14a) and (b) virtually the same response can occur even when the coleoptile is covered with a black cap (Figure 8.14b). Overall, greater progress has been made on the signal perception systems for light and gravity than on how the signals are translated into altered growth patterns.

8.2.6 Nastic movements

Nastic responses differ from tropisms because the direction of movement is not related to the stimulus direction but is instead dictated by the plant. Many legumes with divided leaves such as *Leucaena* (Figure 8.15), *Phaseolus* beans, and the pasture species Siratro (*Macroptilium atropurpureum*), widely grown for forage in Queensland, are good examples. Early in the morning on hot days, leaflets are oriented horizontally, but as temperature and solar radiation levels rise the leaflets move to a vertical position perpendicular to the sunlight. This is helionasty, which cuts down radiation absorption and consequently reduces water use and overheating. When solar radiation declines towards dusk, leaflets return to their former horizontal position. In legumes, movement is controlled by reversible turgor changes in a small fleshy elbow, the pulvinus, located at each leaflet or pinnule base, which can flex back and forth as water flows in or out of the pulvinus cell vacuoles.

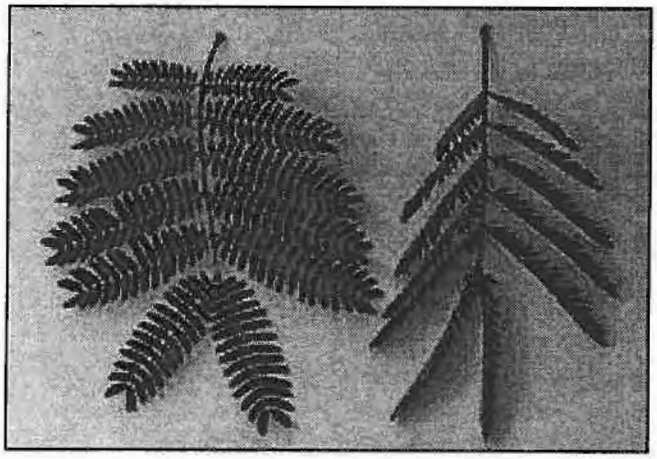


Figure 8.15 Turgor-based nastic movements of leaflets of pinnate bean legume leaves. Left, horizontal leaflets of *Leucaena* early in the day. Right, leaflets folded to Vvertical at mid-day , with leaflets edge-on to the sun
(Photograph courtesy C.G.N. Turnbull)

(a) Seismonasty

Seismonastic or thigmonastic movements are rapid responses to vibration, touch or flexure. Examples are the high-speed bending of leaf pulvini in the sensitive plant *Mimosa sensitiva* (Figure 8.16), and the curvature of hairs of insectivorous plants. In the case of the Venus fly trap, sensory hairs coupled to an electrical signalling system require stimulation at least twice within a 30 s period (Simons 1992). This appears to allow the plant to discriminate single pieces of debris from an insect crawling within the trap. Most seismonastic movements result from the explosive loss of water from turgid 'motor' cells, causing the cells temporarily to collapse and inducing very quick curvature in the organ where they are located.

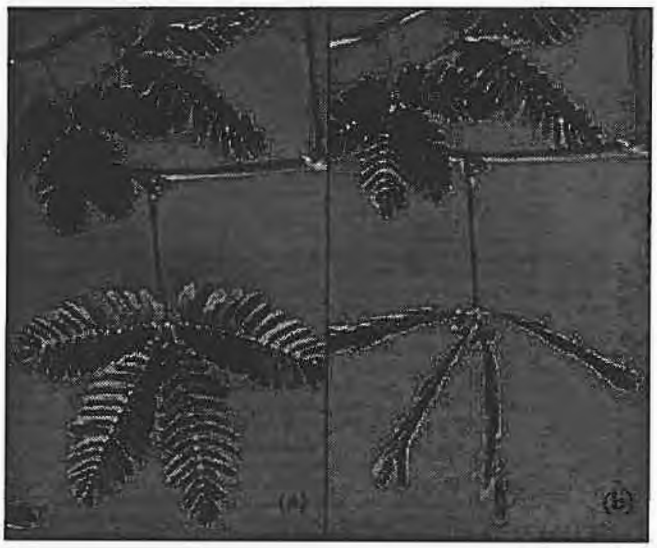


Figure 8.16 Seismonastic movement of pinnae and pinnules in leaves of the sensitive plant (*Mimosa sensitiva*) (a) before and (b) after touch stimulation
(Photographs courtesy J.H. Palmer)

(b) Nocturnal 'sleep' movements

Leaves and leaflets that become vertical at night are called nyctinastic. This is commonly termed a 'sleep' movement, although these plants do not actually slow down their metabolism at night. The 'Prayer Plant' (*Maranta*) is a good example (Figure 8.17). Sleep movements are either growth based, and therefore cease at leaf maturity, or are caused by reversible turgor changes in the pulvinus.

Turgor-based pulvinus flexure

Turgor-based sleep movements are exhibited by many legumes. Examples are clover (*Trifolium*), bean (*Phaseolus*), *Bauhinia*, Coral tree (*Erythrina*) and many tropical legume

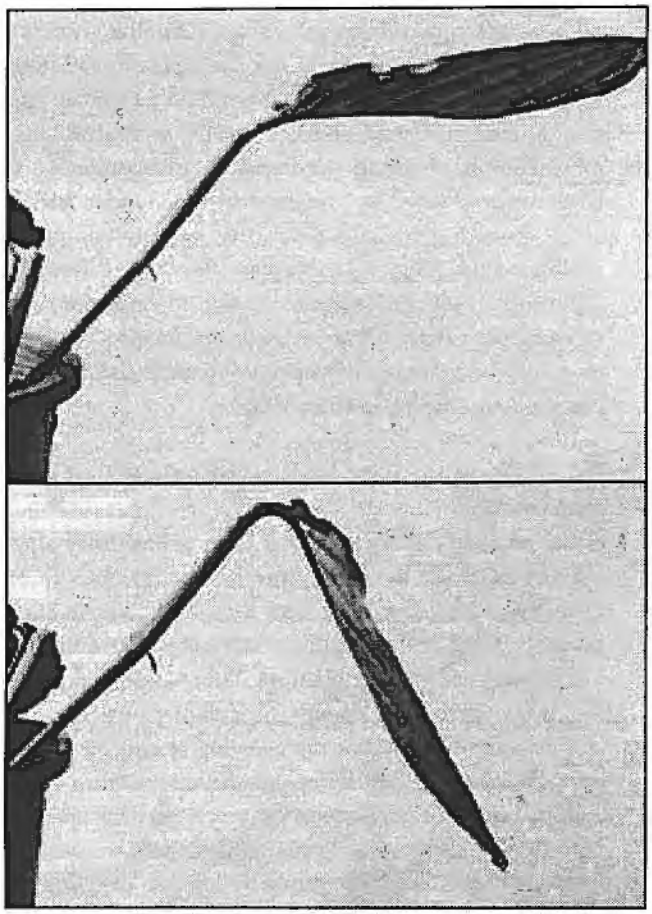


Figure 8.17 Leaf movements in the 'Prayer plant' (*Maranta bicolor*), an ornamental house plant. (a) Leaf lamina in a near horizontal daytime position. (b) Leaf inclined down into night-time position. The leaf movement is caused by turgor changes in the fleshy pulvinus at the base of the leaf blade
(Photograph courtesy J.H. Palmer)

Figure 8.18 Growth-based epinastic curvature in sunflower petioles. The plant on the right was exposed to 20 μg of ethylene in the surrounding air for 10 h. The epinastic curvature of the petioles is due to growth of cells in the upper half of the petiole being strongly promoted by ethylene, causing the upper half to increase in length and induce the observed downward curvature of the petioles. Older leaves at the base of the plant have ceased growth and hence their petioles do not respond to ethylene
(Photograph courtesy J.H. Palmer)

trees, such as *Pithecelobium saman* and *Leucaena*. Turgor-based sleep movements occur mainly in compound leaves with a mechanism similar to helionasty. The daily rhythm of water movement results from a flux of potassium ions from one side of the pulvinus to the other, either increasing or decreasing the water potential of cell vacuoles in each half.

Growth-based petiole epinasty

Other species follow a daily rhythm of leaf movement due to differential growth of upper and lower halves of the petiole. The day–night rhythmic curvature of the petiole is not related to a directional stimulus and is termed 'epinastic'. Like turgor-based 'sleep' movements, magnitude varies with the amount of solar radiation intercepted. Epinastic growth movements may be caused by diurnal changes in production of the plant hormone ethylene, which promotes growth of cells on the upper side of the petiole, inducing downward curvature (Figure 8.18). Leaves constantly produce small amounts of ethylene and, according to one hypothesis, production increases towards the end of the day, moving the lamina from horizontal to vertical. The opposite would occur towards the end of the night, allowing the lamina to return to the horizontal daytime position. Supporting evidence comes from petiole cells where ability to respond to ethylene is blocked by silver thiosulphate, and the epinastic leaf movement subsequently disappears.

Nocturnal leaf folding may help plants to conserve water by promoting dew formation, since the air and soil beneath the canopy cool more rapidly after the canopy has folded up or become vertical. The lower temperature then promotes dew development, which falls to the ground around the base of the plant, supplementing rainfall.

Growth-based epinasty is also seen in many dicotyledonous seedlings during germination, when the end of the shoot is bent over in a plumular hook. The hook is a temporary structure which protects the apical bud as the shoot pushes through the soil. It is created by cells on one side of the plumule expanding more rapidly than cells on the opposite side, possibly in response to ethylene, which is produced by the plumule in darkness. On reaching the soil surface, the plumule is exposed to daylight which appears initially to reverse and then to cancel the differential response to ethylene, and consequently the stem straightens.

8.3 Reproduction

8.3.1 A time to flower

Survival of many plant species depends on setting seed well in advance of seasonal environmental extremes including frost,

heat or drought and particularly during pollen formation and pollination. Synchrony of flowering is also beneficial especially for outbreeding species which must time their reproduction to coincide with flowering of other individuals or genotypes and often with the presence of insect and bird pollinators. The natural light and temperature environment provide much of the seasonal information essential for control of flowering time, but plant age or maturity can also be important.

(a) Plant maturity and flowering time

Many plants grow vegetatively for periods ranging from weeks to years and then flower autonomously, apparently without identifiable environmental control. Flowering of 25–30-year-old bamboo is one such example: no environmental cue is known for this species. Perhaps it has its own built-in developmental clock which determines flowering time as in some annuals which flower autonomously. In contrast, other species may flower late due instead to inappropriate cultural or environmental treatments. In this instance, flowering may not occur irrespective of whether the juvenile phase has ended.

In some species, flowering occurs after the apex has produced a particular number of leaves. This apparent leaf counting may reflect an interplay between older leaves and the roots. In tobacco, for instance, proximity of the roots to the main shoot apex is critical. Plants remain vegetative until the shoot apex is more than five to seven leaves above the roots or above a zone of experimentally induced root formation on the stem (McDaniel 1980).

Extremely fast flowering without any apparent juvenility is seen in some desert annual plants. They may germinate and reproduce rapidly after rainfall, forming as few as two or three leaves and then flowering. The terminal shoot apex and all axillary apices may become floral. More often, however, such rapid flowering is restricted to either lateral or terminal meristem(s), leaving a second population of meristems available for further growth and reproduction if favourable conditions persist (Hayashi *et al.* 1994).

With some agricultural crops bred for earliness of flowering, such as soybean and rice, early maturity may have resulted from a shortening of the juvenile phase (Evans 1993) rather than from changes in sensitivity to environmental cues. Thus, for some crop plants, duration of juvenility can influence chronological and developmental time from seed germination to flowering, regardless of other physiological controls of flowering.

As an adaptation for survival, juvenility is an advantage and a single gene controlling its duration is known in *Pisum* (Murfet 1985). Embryonic flowering (*emf*) may peform a similar role in *Arabidopsis*. As discussed later, several other floral-specific genes also influence aspects of this floral transition. In contrast to the abbreviated juvenile phase of annuals, perennials such as apple or mango have a juvenile phase often lasting five to eight years. Various cultural and environmental manipulations including drought, nitrogen fertilisation, stem girdling, grafting and CO_2 enrichment can reduce this period in conifers (see Pharis and King 1985). The juvenile period of some *Eucalyptus* species can also be shortened from two to three years to 9–12 months if grafted cuttings are exposed to cool inductive conditions and treated with an inhibitor of gibberellin biosynthesis. Endogenous gibberellin A_1 (GA_1) levels were lowered by this treatment (Moncur and Hasan 1994) so high gibberellin levels may be one component of prolonged juvenility in *Eucalyptus*. We will see later that in other species gibberellins may *promote* flowering, so we need to make clear distinctions between species, process (breaking juvenility or inducing flowering) and even the type of gibberellin (see Pharis and King 1985).

(b) Flowering time and environment: photothermal input

Environmental factors that limit plant growth may also profoundly influence flowering time. Suboptimal growth conditions may delay flowering and give an apparent extended juvenile phase, and often light intensity, light duration and temperature are major limitations. Thus, a summation of both inputs (the photothermal sum) over all or part of the calendar year helps to characterise the growing season. Photothermal sums indicate whether there is adequate time from sowing to seed maturation for an annual crop or wild plant species. The yearly cycle of solar radiation highlights how this varies with latitude (Figure 8.19). There are losses due to cloud and to atmospheric interception. Of the remaining sunlight, the visible/photosynthetic component is about 45% and the rest is 'heat'. The calculation of photothermal units integrates these heat and visible light inputs. For example, although daily photosynthetic flux at extreme latitudes may be high in summer, the growing season is extremely short.

Thermal sums (based on a heat sum above a 10°C base) have been used in the USA to predict the likely penalty in flowering time, and hence in yield, from growing long-season (late flowering) corn varieties at a higher latitude (Figure 8.20). To maintain yield, breeders have had to obtain lines with shorter growing seasons, in this case selecting varieties with more rapid early seedling growth and therefore requiring

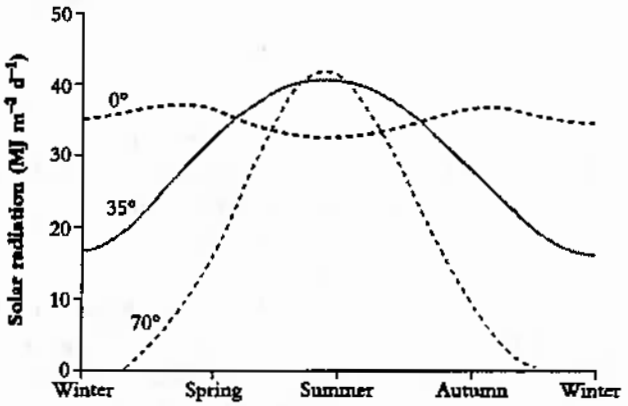


Figure 8.19 As the seasons change, solar radiation incident on the earth can fluctuate dramatically at extreme latitudes or very little at the equator (Based on Gates 1962)

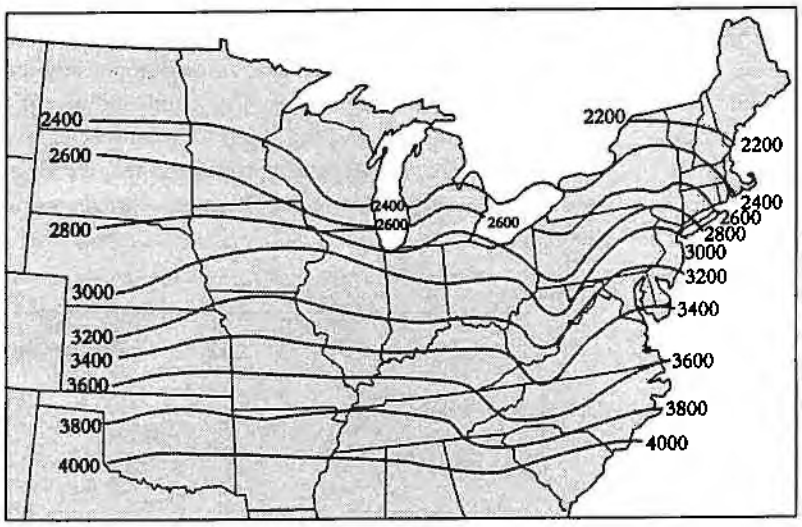


Figure 8.20 Heat sums for corn growth have been computed from 30 years of temperature records for the corn belt of the USA. The numbers represent the cumulative amount of heat above a 10°C base (the nominal lower limit for growth) over a whole growing season. Varieties for the colder zones need to be fast growing and require a smaller heat sum if they are to yield well
(From Newman 1971)

smaller thermal sums. Similar approaches with other crops such as soybean have used data from analysis of field environments and controlled environment studies (see Evans 1993).

Photothermal responses for perennial crops are more complex, partly because flowering may relate to current *and* previous years' environmental conditions. Controlled environment experiments help us unravel some of the interactions. In vines such as grape and kiwifruit, the extent of bud dormancy can be determined on cuttings taken from 'winter' canes and transferred to controlled environment cabinets. This enables prediction of timing of field budburst for each cultivar (see Section 8.1.3).

Another approach with perennial plants involves collection of field flowering and temperature data over a number of years at different latitudes. For two ericaceous shrubs a heat sum model predicted flowering times at eight field sites in Canada (Reader 1983) and similar heat sum relationships have been shown for another 15 species at 200 latitudinal sites in Alberta. The earliest spring flowering species had the smallest heat sum for flower opening.

Information on climate and plant responses to the environment provides one way to estimate global reproductive potential. In equatorial zones, temperature and irradiance change less over the year (Figure 8.19) and time of flowering may instead reflect seasonal rainfall patterns. In warmer temperate zones, early spring flowering and adaptation to intermediate heat sums can ensure reproduction prior to high summer temperatures and drought stress, but a second favourable climatic window is autumn. At high latitudes or at altitude, growth and flowering occur during midsummer.

Although these ideas can explain seasonality of flowering, photothermal relationships match best to the period of development up to flower opening (Reader 1983). They apply less well to floral induction, which is often a response to specific episodes of high or low temperature and/or to seasonal change in daylength. Assessment of such responses is best studied in controlled environment chambers where each component can be varied independently. In this way we can

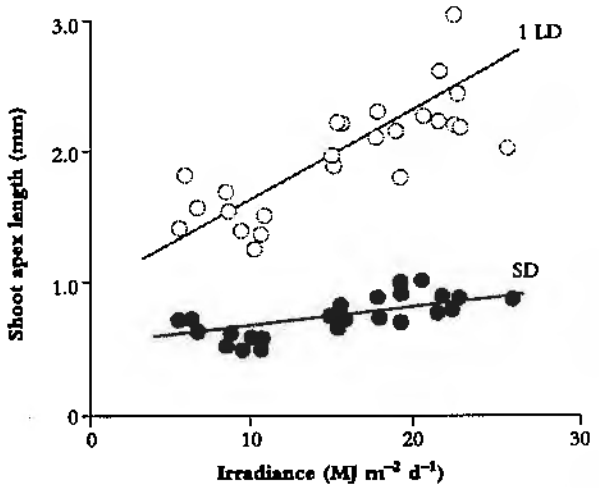


Figure 8.21 Effect of seasonally changing total radiation on inflorescence initiation in *Lolium temulentum* growing in a fixed-temperature regime. Plants either flowered after a single long-day (1LD) exposure given at different times of year or remained vegetative in short days (SD). *L. temulentum* is a long-day plant with apex length ≥ 1 mm indicating transition to a floral state, measured 21 d following floral induction
(Based on King and Evans 1991)

reveal effects on flowering of seasonal changes in amount and duration of daylight, the 'photo' component of photothermal responses. As shown in Figure 8.21, flowering response of the grass *Lolium temulentum* varies with irradiance at the time of exposure to a single inductive long day. Increase in photosynthetic input is beneficial but is not the major limiting factor for flowering. Rather, daylength (photoperiod duration) is the major determinant of flowering in this and many other species.

(c) Daylength and flowering time

As long ago as 1914, scientists recognised that daylength regulated flowering time of hops (*Humulus japonicus*) and by 1920 two Americans, Garner and Allard, had demonstrated daylength control of flowering of many species. They termed the species either short- or long-day plants (SDPs or LDPs). SDPs flower in response to a decrease in daylength, that is, an

increasing length of the daily dark period and a shortening photoperiod; LDPs flower in response to increasing photoperiod. As well as causing flowering, daylength can also influence winter dormancy of buds, tuberisation, leaf growth, germination, anthocyanin pigmentation and sex expression.

Change in daylength is identical from year to year (Figure 8.22) and so provides precise information on season. Thus a photoperiodic plant can time reproduction to avoid midsummer drought, autumn cold or late spring frosts. Summer flowering at higher latitudes typically will involve a response to long days. In the tropics, daylength changes little, so selection pressure could be for daylength insensitivity or short-day response, provided plants could measure such small changes in daylength. Withrow (1959) calculated that to measure seasonal time to within one week required a 1–3% precision in measurement of daylength. Only a 4–12% precision was required for accuracy to within a month. In the tropics, a 1–3% accuracy would mean distinguishing photoperiods differing by 7–21 min around a 12 h daylength. Remarkably, several species including some tropical plants do show such accuracy. In studies with rice, a tropical SDP, flowering occurred 30 to 50 days later when the photoperiod was increased by only 10 min, from 11 h 50 min to 12 h (Dore 1959).

Detection of daylength involves a photoreceptor called phytochrome. This pigment detects very low energies of visible light, especially red and far-red wavelengths. The consequence is that major daily and seasonal fluctuations in photosynthetic light intensity do not influence measurement of daylength. So sensitive is phytochrome that at latitudes up to 40°, plants respond to twilight radiation for about 20 min after sunset and before sunrise (Salisbury and Ross 1983). At high latitudes, the midsummer sun may never set as far as phytochrome sensing is concerned. We return to discussions of phytochrome in Setion 8.4.

The duration of daily light/darkness which is effective for flowering may be very precise or very broad. Such contrasting patterns are illustrated in Figure 8.23 along with typical long-day, short-day, intermediate, ambiphotoperiodic or day-neutral (indifferent) responses. Daylength-indifferent types represent less than 15% of the 150 or so grass species reviewed by Evans (1964), although this proportion may be an underestimate as 'observed' day-neutral responses might not always be reported.

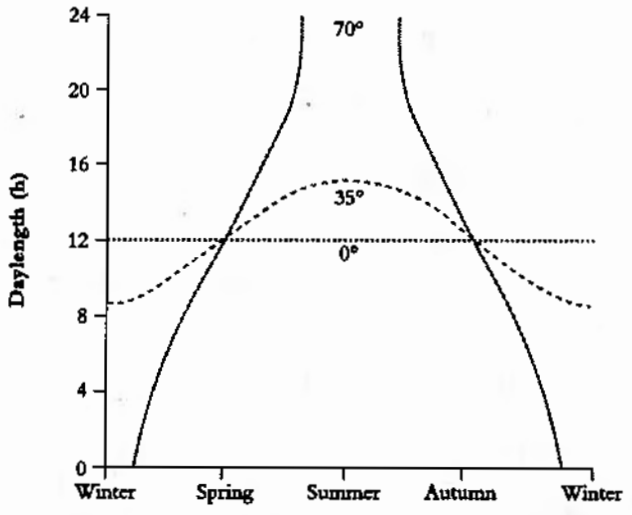


Figure 8.22 Seasonal daylength at various latitudes. Values at other latitudes fit between those shown
(Redrawn, with permission, from Salisbury and Ross 1983)

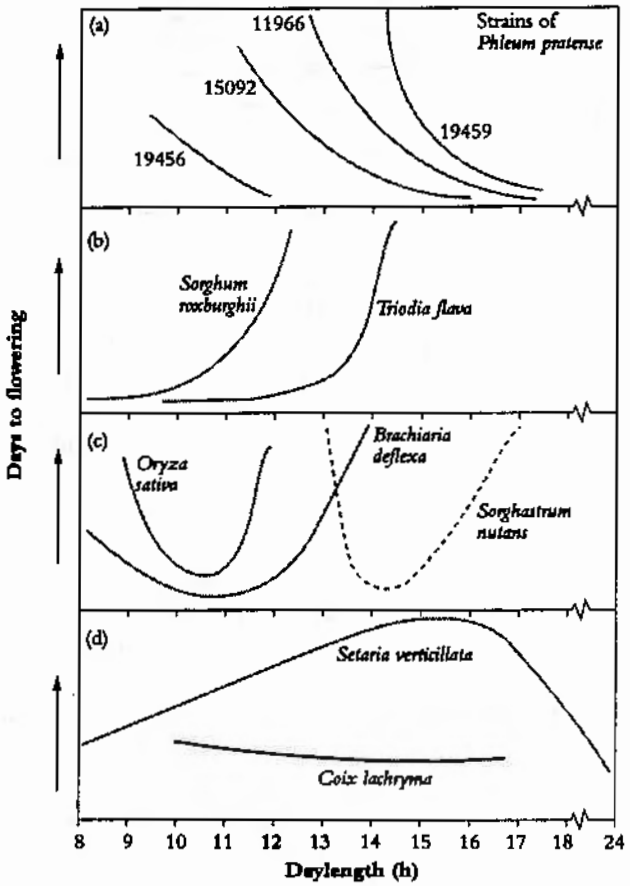


Figure 8.23 Control of flowering by daylength in (a) several strains of a long-day grass, (b) two short-day grasses, (c) three intermediate-day grasses, (d) a daylength-indifferent and an ambiphotoperiodic grass
(From Evans 1964)

Within a species there can be large differences in photoperiod response, as in the LDP *Phleum pratense* (Figure 8.23). The full range of daylength response types may even be found within a single species. For example, in a controlled environment study of 30 ecological races of the Australian grass *Themeda australis*, Evans and Knox (1969) found that low-latitude strains, from 6° to 15°S, behaved as SDPs (Figure 8.24). Races from more southerly origins to 43° were LDPs with some responsive to vernalisation (see later). This ecotypic variability exemplifies heritability and adaptability of environmentally responsive flowering and appears to have aided reproductive success of *Themeda*. If the species migrated to Australia via Asia and New Guinea, it would probaly have adapted from a short-day response to day neutrality or sensitivity to long day and to vernalisation.

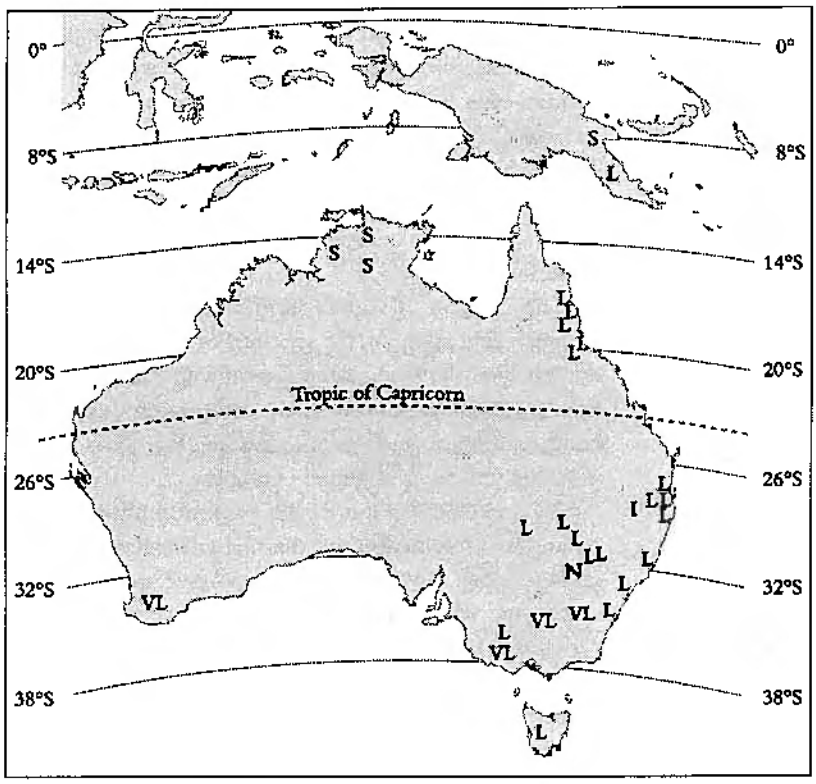


Figure 8.24 Effect of site of origin of clones of *Themeda australis* on their flowering in response to daylength (S = short day, L = long day, N = neutral, I = indifferent) or to vernalisation (V)
(From Evans and Knox 1969)

Some plants will flower after just one cycle of the appropriate daylength. Cocklebur (*Xanthium strumarium*) and Japanese morning glory (*Pharbitis nil*) are classic examples of SDPs responding to one short day, or more correctly, one long night. Similar single-cycle responses are found for LDPs such as *Lolium temulentum* (Figure 8.21). Other species require several days (e.g. soybean, strawberry) or weeks of exposure to the appropriate daylength (e.g. Geraldton wax, chrysanthemum). In some plants a sequence of short days must precede long days (SLDP) as for some clovers (e.g. *Trifolium repens*) and grasses (e.g. *Poa pratensis*). Conversely, some species respond as long–short-day plants (LSDP) including *Aloe*, *Bryophyllum* and some mosses and liverworts (see summaries in Lang 1965; Vince-Prue 1975). Some dual photoperiodic responses may be satisfied simultaneously so that flowering is best at intermediate daylengths (e.g. some sugar cane genotypes). The converse is also known, ambiphotoperiodic response, with best flowering at either short or long days but not at intermediate daylengths (Figure 8.23). Separation in time occurs in some grasses which respond to short days for primary induction leading to a microscopically visible inflorescence but later to long days for subsequent development to anthesis (Heide 1994).

(d) Low temperature and flowering time

Vernalisation responses

Although growth is limited by low temperature, scientists in the mid-nineteenth century recognised that floral initiation of many species requires exposure to cold. For a temperate cereal such as wheat, low-temperature exposure of imbibed grain caused winter lines to flower like their spring wheat counterparts. We term this response *vernalisation*, meaning 'to become spring-like'.

Vernalisation-responsive species include winter annuals, biennials and perennials. Many are also LDPs including some grasses and species with a rosette growth habit. Effective temperatures for vernalisation range between -6°C and 14°C, with most temperate species responding best between 0°C and 7°C. In all cases, these temperatures are below those optimal for growth. Floral primordia are sometimes initiated during the cold period, as in brussels sprout, turnip, stock and bulbous iris. Alternatively, cold treatment is a preparatory phase enabling later initiation of flowers.

Generally, prolonged exposures of one to three months are required for vernalisation but this varies with temperature and species. However, as with photoperiodic species, some respond to a single cold day, for example chervil. In *Geum*, the vernalisation period depends on meristem location, ranging from two to three months in axillary meristems to one year for the terminal apex. Heterogeneity of floral response of meristems has clear adaptive benefits, whether for perennation as with *Geum* or for opportunistic responses to rainfall as for desert ephemerals (see above).

As with photoperiodism, dependence of flowering on vernalisation changes with latitude. For example, a vernalisation response appears only in high-latitude ecotypes of *Themeda australis* (Figure 8.24) and is likewise more important for species and ecotypes from higher altitudes. European thistle (*Cirsium vulgare*) collected from the Mediterranean to Scandinavia exhibit vernalisation requirements predominantly

Table 8.8 Vernalisation (low-temperature) responses can occur in pea (Pisum sativum) where the shoot apex was not cold treated. Scion–stock graft pairs were scored for node position of first flower. Either or both or neither of each pair were cold treated prior to grafting, and were compared with intact cold-treated and warm control plants. The earlier flowering in the majority of the plants in the warm/cold combination indicates a graft-transmissible signal due to cold perception in the cotyledons or the root

	First flowering node
Intact plants	
Warm	34–50
Cold	9–24
Grafted plants (scion/stock)	
Warm/warm	32–46
Cold/cold	12–24
Warm/cold	12–16 (62%) or 30–48 (38%)*
Cold/warm	18–22

(From Reid and Murfet 1975)
Nodes are numbered from base of plant: where two zones flowered, the percentage of plants in each category is shown in brackets.

in lines from colder, more northerly sites (Weselingh *et al.* 1994). In addition to latitude effects in the grass *Phalaris aquatica*, there is a superimposed altitudinal cline.

Leaves sense photoperiod, but perception of low temperatures resulting in vernalisation responses can be by the shoot apex instead. Chilling of leaves is usually ineffective (Bernier *et al.* 1981). However, cold-treated leaf cuttings of species such as *Lunaria* and *Thlaspi arvense*, and even chicory root explants, regenerate plants which flower without further vernalisation (Metzger 1988). One hypothesis is that vernalisation responses may be initiated only at sites with potential for cell division, that is, meristems or regenerating tissues. On the other hand, in pea and sweet pea, there is clear evidence of transmission of vernalisation signals across graft unions (Table 8.8). In these experiments, perception of cold must have occurred in cells other than those in the responding shoot apex. These species also exhibit normal shoot apex vernalisation responses, so there can be two different mechanisms of low-temperature sensing.

The presence of water and metabolic activity are essential requirements for vernalisation. We deduce this from vernalisable species which can respond during seed germination. Radish seed, for example, cannot be vernalised when dry or in a nitrogen atmosphere.

The vernalised state is quite stable in seeds of some species: they can be dried after cold treatment, even stored for long periods, and then sown without loss of response. However, particularly with marginal vernalisation, temperatures immediately following often need to remain below 25°C to prevent devernalisation. High temperature up to 40°C for a few days sometimes annuls a preceding cold exposure (Bernier *et al.* 1981). Indeed, devernalisation every summer may reset the flowering of perennial plants so that they require renewed vernalisation each winter.

Photoperiod requirements post-vernalisation are diverse. Many winter annuals or biennials require long days following vernalisation. For example, vernalised *Hyoscyamus* will not flower under short days but under long days promptly forms flowers, even with 300 short days between vernalisation and induction. In contrast, sensitivity of spinach to inductive long days is altered following cold treatments with a shortening of the critical day length from 14 h to 8 h. A few cold-responsive plants, such as chrysanthemum, require short days after vernalisation.

The genetics of vernalisation range from simple to very complex depending on the species. For example, a single locus distinguishes the biennial, cold-requiring strain of *Hyoscyamus* from its annual counterpart. By comparison, vernalisation of hexaploid wheat involves at least three loci (Vrn 1, 3 and 4), probably reflecting its genetic complexity.

Pea and *Arabidopsis* normally respond both to photoperiod and to vernalisation. Of the many late-flowering mutants known, some are vernalisation responsive, including *gigas* (*gi*) in pea and *luminidependens* (*ld*) in *Arabidopsis*. There are also vernalisation-unresponsive and early-flowering mutants. One simple explanation is that the wild-type products of some of these genes are inhibitors of floral induction or initiation or, conversely, stabilise vegetative growth.

Vernalisation may involve decreased DNA methylation allowing activation of suites of genes including some involved in synthesis of gibberellins. For example, extending the earlier work of Hirono and Redei (1966), Burn *et al.* (1993) found that vernalisation-responsive late-flowering mutants of *Arabidopsis* treated with the demethylating agent 5-azacytidine flower earlier than unvernalised controls. From this result, they concluded that demethylation occurs during vernalisation and leads to selective derepression of genes required for flowering.

Cool temperature response

In addition to classic vernalisation responses, there are many reports of species, especially from warm climates where near-freezing temperatures are infrequent, which flower if exposed to temperatures from 10°C to 20°C. For some tropical fruit crops (e.g. mango, avocado, lychee, longan), especially those grown in the subtropics (latitude 23°–30°) where substantial seasonal temperature changes occur, floral induction results from exposure to night temperatures of 10°–15°C. Because tropical species are relatively under-researched compared with their temperate counterparts, physiologists have yet to decide whether these cool responses have similar mechanisms to temperate vernalisation but are adapted to a different temperature range. Another possibility is that flower initiation and development are blocked/reversed by higher temperatures, so low temperature could merely be a passive condition permitting expression of an innate capacity to flower. This may be the case for *Acacia* and rice flower (see King *et al.* 1992) but for *Pimelea ferruginea*, which flowers if exposed to temperatures below a daily average of 16–18°C for five to seven weeks, the response is inductive and higher temperature does not cause loss of developing flowers (King *et al.* 1992).

(e) Water stress and nutrition

In some species including *Lolium*, *Pharbitis* and *Xanthium*, floral induction and development are blocked by water stress (see Bernier *et al.* 1981). For *Lolium*, an 8 h stress inhibited flowering only if given at the time of the long day, not one day before or after. Shoot apex abscisic acid (ABA; see Chapter 9) content increased transiently up to 10-fold in association with the brief water stress (King and Evans 1977). Furthermore, ABA inhibited flowering if applied at the time of the long day. Later in flower development, water stress or ABA application can result in sterility in wheat. The problem is morphologically aberrant pollen, but seeds are still set if plants are hand pollinated (Morgan 1980).

By contrast, positive responses of flowering to water stress are also known. For the geophyte *Geophila renaris*, growth under water-limited conditions for two months causes flowering (see Bernier *et al.* 1981). Similarly, water stress coupled with enhanced photosynthetic conditions, high temperature and gibberellin application can cause precocious flowering in some conifers (Pharis and King 1985). In mango trees grown in the tropics with little temperature variation, seasonal flowering appears to be promoted by water stress during the dry season. This may relate to trees having an extended period of suspended growth during which ability to flower gradually develops, for example as a result of accumulation of stored carbohydrate.

Nutritional status of plants has little direct influence on floral initiation, although in many species there are effects on flower number and on fruit and seed development. For example, pollen fertility in wheat is reduced by excesses and deficiencies of trace elements including copper and boron (reviewed by Graham and Nambiar 1981). In strawberry, plant size and fruit and flower number increase as nitrogen supply is increased (Guttridge 1969), but the supply of nitrogen during early stages of flower initiation may enhance vegetative growth not flowering. Such complex responses make it difficult to argue that transition to flowering requires low-nitrogen status coupled with enhanced carbon supply. Numerous studies have failed to demonstrate an inverse relationship between nitrogen supply and flowering and, as noted above, there are often positive effects on floral development (see Bernier *et al.* 1981). Perhaps a unique response to nitrogen is the dramatic increase in flowering of apple supplied with nitrogen but only if supplied as ammonia (Grasmanis and Leeper 1967). Overall, mineral nutrients, while essential for growth, may not specifically regulate flowering.

(f) Environmental and seasonal synchronisation of flowering

The species in its natural environment

Control of seasonal flowering time may be as simple as the acquisition of a long-day or short-day photoperiodic response, or to both as in LSDP where exposure first to long summer days is essential to guarantee flowering in the short days of autumn. Alternatively, floral development may occur in spring when both temperature and irradiance increase rapidly to permissive levels (Figure 8.19). A vernalisation requirement allows for spring flowering, or for summer flowering when combined with a long-day response.

Often, a combination of short day then long day, as well as temperature, is important in synchronisation of flowering of perennial grasses (Heide 1994). Comparison of environmental tolerances of *Bromus inermis*, a species adapted to lower latitudes, and *Poa pratensis*, an arctic–alpine species, highlights how these inputs determine survival. For flowering, both species require short-day or low-temperature exposure followed by long days. The short-day response is strict in *Bromus* and, because of intolerance to low temperatures, it will never flower at the high latitude of Tromsö (69°39′N), as shown by its climate phototherm (Figure 8.25). The response of *Poa*, by contrast, overlaps an arctic phototherm (Tromsö) but this species is intolerant of the higher summer temperatures at lower latitudes. Dual induction responses also enable high-latitude-adapted species to initiate inflorescence primordia in autumn short days. The outcome is to maximise the number of summer days available for seed development because anthesis proceeds rapidly in the following summer long days, even in the short, cool arctic growing season.

Field to nursery transplantations have often demonstrated environmental influences on flowering, as noted above for vernalisation of *Girsium arvense*. Alternatively, controlled environment studies of the type used by Evans and Knox have revealed ecotypic adaptation of flowering in *Themeda* (Figure 8.24). Rarely have the two approaches been combined. Either photothermal models have been used to assess field flowering data or laboratory environmental response profiles have been

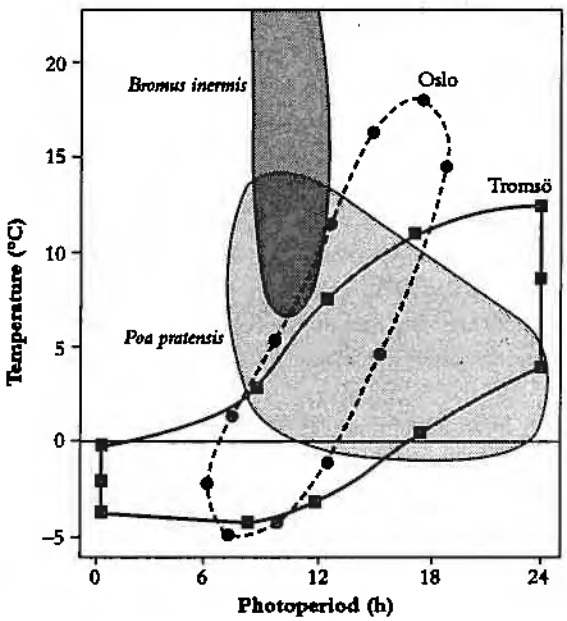


Figure 8.25 Climate phototherms for Tromsö (69°39′N) (——) and Oslo, Norway (59°55′N) (– – – –). Mean monthly temperature versus photoperiod together with optimum areas for primary induction of flowering of *Bromus inermis* and a high-latitude species *Poa pratensis*
(Redrawn, with permission, from Heide 1994)

incorporated into empirical models predicting field response. However, with *Pimelea ferruginea* grown simultaneously in controlled environments and in the field over winter (King *et al.* 1996), there was a close match between effective temperatures for flowering in the field and laboratory. In addition, evidence for adaptation to small (4°C) temperature differences came from a high-latitude ecotype from 31°S which was unable to flower when transplanted to the warmer extreme of the species distribution (28°S).

The environment and predicting flowering time of field crops

Phototherms only broadly define the tolerance of a species to its environment. A more definitive approach uses rates of response of flowering to photoperiod and temperature based on constants derived from controlled environments. Threshold limits are also imposed to constrain models to response envelopes of the sort illustrated in Figure 8.25. Six crop species (soybean, cowpea, mungbean, chickpea, barley and lentil) sown at different latitudes and times flowered in the field at times which correlate well with those predicted from a simple linear additive model (Lawn *et al.* 1995). However, no allowance is made for effects of light intensity and extreme conditions outside the threshold limits which can be important for flowering, for example vernalisation or warm temperatures.

The environment and commercial nursery floriculture

Prior information on environmental response has been crucial to nursery production of potted flowering plants including the SDPs chrysanthemum and poinsettia. However, there may be inevitable compromises in some of the complex protocols required for commercial production of an Australian SDP, Geraldton wax. Its critical photoperiod is about 13 h, so the maximum tolerable daylength would be about 12 h from sunrise to sunset plus 20 min each pre-dawn and twilight (Dawson and King 1993). Thus, in summer, glasshouse blackout curtains are used to maintain the inductive short day, but this is obviously not an option for field-grown plants. Glasshouse summer temperatures exceeding 35–40°C, well above the optimum for the species, are another problem. As a comparison, optimal mean daily temperature for chrysanthemum is about 21°C (Pearson *et al.* 1993). Consequently, greenhouses are often shaded to avoid costly cooling, but then lower photosynthetic input may result in poorer flowering.

Flowering of woody horticultural species

Prolonged juvenility of woody species is a problem for growers and breeders of tree and vine crops. However, there are so many uncontrolled variables in the field that it can be difficult to identify the inductive factors. Yields can be severely depressed by inappropriate timing of practices such as pruning, irrigation and fertilisation. Furthermore, inductive conditions may be required for several months. One solution for mango, lychee, olive and citrus has involved the use of controlled environments and 'mini' plants grown from cuttings. These showed that cool temperatures were required for induction, a response similar to *Pimelea* and many other ornamental and woody species.

For some species, microscopic examination of shoot meristems has augmented our ability to make decisions on practical management of flowering. For example, in kiwifruit (*Actinidia*) and stone fruits (*Prunus* spp.) floral induction occurs in the previous growing season, whereas in most subtropical species no initiation takes place until winter. In the case of kiwifruit, it was discovered that late summer pruning was removing many of the floral apices (Snowball 1995).

Clearly, knowledge of environmental effects on flowering has been essential for development of nursery, orchard and agricultural crops. Particularly for field crops, breeders have selected for day-neutral responses. For glasshouse crops, genotype and environment have often been altered. The future offers many opportunities for applying our knowledge of daylength and photothermal responses.

(g) Summary

Plants depend on natural daylength changes (e.g. short day, long day, short day→long day, long day→short day and/or low temperatures to regulate timing of reproduction. Progressively shorter days in autumn, for example, are likely to cause flowering in LSDPs. A requirement for low temperature (vernalisation) can ensure bienniality in spring-germinating species. Many warm-adapted species appear to depend on cool rather than cold temperature for spring flowering.

8.3.2 The processes of floral induction and initiation

Following discovery of photoperiod-regulated flowering, there soon followed evidence of leaves as photoperiod sensors, of a timekeeper involving endogenous circadian rhythms, of transmissible florigenic signals and of a resulting cascade of developmental changes at the apex.

Although sometimes used loosely, it has long been clear that the term 'flowering' embraces an amazing series of signalling systems and developmental transitions. Photoperiodic induction refers to photoreceptor-driven, leaf-specific processes. Flower initiation at the apex is now divided into floral evocation and floral differentiation; evocation describes the early processes occurring at the apex before irreversible commitment and differentiation of flower primordia. Although the term 'florigen' was coined initially, there may be multiple transmitted florigenic stimuli so 'floral stimuli' or 'florigens' are more appropriate.

(a) Photoperiod and leaf photoresponse

Sensing of photoperiod requires photoreceptor pigments and a responsive organ. Elegant experiments involving selective light exposure of different parts of the plant confirmed that

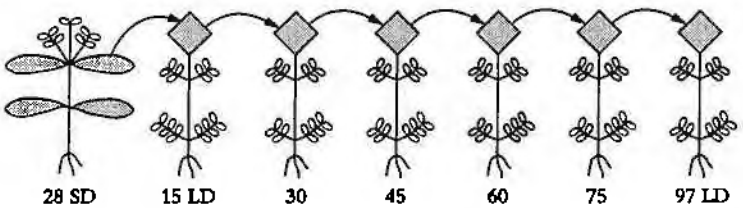


Figure 8.26 A permanently inductive state can be demonstrated for leaves of some photoperiodic species. After 28 days of short days (SD), a leaf of *Perilla* returned to long days (LD) will continue to produce graft-transmissible flowering stimulus for at least 97 days, involving six successive grafts of the same leaf to vegetative, long-day-grown receptor plants
(From Zeevaart 1958)

Table 8.9 *For* Lolium temulentum, *a single daylength extension of 16 h with incandescent (inc) or low or high fluorescent (fluor) illumination determines the extent of inflorescence initiation (apex length). Initiation can occur with or without a change in apex sucrose content*

			Flowering response		
Inductive treatment	**Photoperiod extension**	**Extension PFD (μmol m^{-2} s^{-1})**	**Apex length (mm)**	**Floral stage**	**Apex sucrose content (% DWt)**
SD	None	0	0.87 ± 0.2	Vegetative	2.65 ± 0.34
1 LD	Inc	11–14	2.20 ± 0.1	Florets	2.86 ± 0.39
1 LD	Fluor	11–14	1.17 ± 0.4	Double ridges	3.41 ± 0.14
1 LD	Fluor	200–250	1.91 ± 0.1	Florets	6.98 ± 0.70

(From King and Evans 1991)

the leaf blade is the photoresponsive site. Defoliated plants show little or no photoperiodic response and direct illumination of the shoot tip is mostly ineffective. A leaf, once photoperiodically treated, may be permanently changed. Leaves of the SDP *Perilla*, for example, exhibit a remarkable permanently induced state to the extent that a single leaf is capable of causing flowering when grafted in sequence to six vegetative receptor plants over a period of 14 weeks (Figure 8.26).

There are at least three plant pigments that could regulate photoperiodic flowering responses: chlorophyll via photosynthesis, phytochrome and the blue light receptor (see Section 8.4). Photosynthetic input will enhance flowering as shown earlier for the LDP *Lolium* (Figure 8.21). Measurements of shoot apex sugars show that increased photosynthetic sucrose supply to the shoot apex may be important, but on its own it is insufficient. The primary requirement is instead for activation of phytochrome (see Section 8.4). For example, *Lolium* can flower in response to a single long day extended with non-photosynthetic light. Far-red-rich wavelengths from tungsten lamps are more effective than red-rich wavelengths from fluorescent lamps (Table 8.9), and this is typical for LDPs. For another LDP, *Arabidopsis*, involvement of phytochrome in flowering is revealed by a brief (10 min) end-of-day exposure to pure far-red (FR) light which promotes flowering with classic R/FR photoreversibility (Figure 8.27). What in perhaps surprising, considering the range of phytochrome mutants in *Arabidopsis*, is that none of the mutants presently known for phytochrome A or B (see Section 8.4) delays flowering (Figure 8.27).

Phytochrome's role in flowering in SDPs relates to increases in the duration of the dark period (Figure 8.28). Light in the middle of the long inductive dark period (a 'night break') inhibits flowering of SDPs — they experience a 'pseudo' long day. Conversely, night breaks may promote flowering of LDPs.

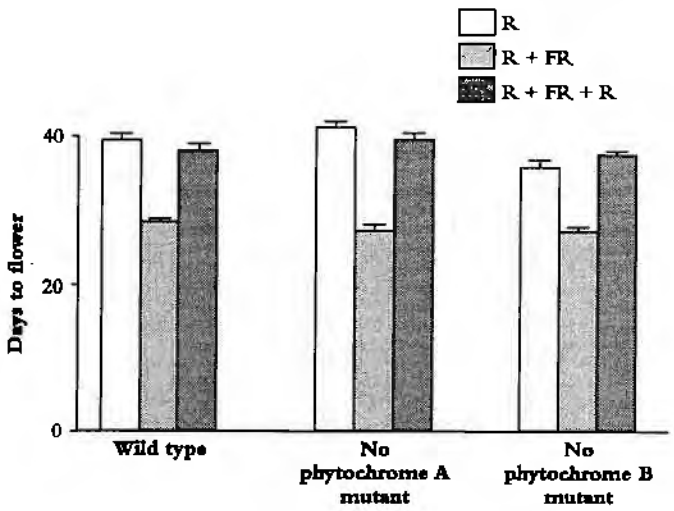


Figure 8.27 Photoreversible, R/FR regulation of flowering in the LDP *Arabidopsis* by either light-stable phytochrome B or light-labile phytochrome A
(Reproduced, with permission, from Bagnall *et al.* 1995)

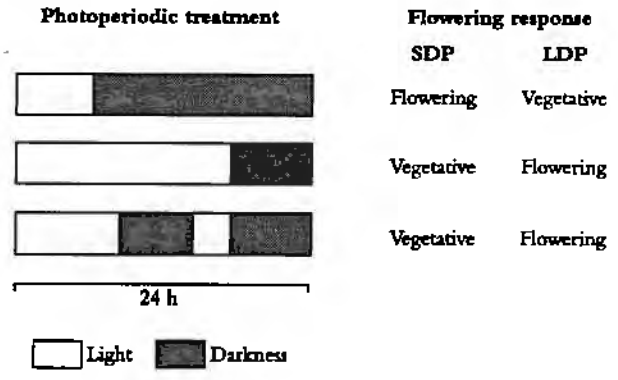


Figure 8.28 Effect of photoperiod and night-break interruption on flowering of SDPs and LDPs. The night interruption may be less than 5 min of very dim light, as in some SDPs, or may require prolonged (1–2 h) exposures, as in some LDPs.

For SDPs, the night-break duration may be amazingly brief (1–300 s) and the response often shows R/FR photoreversibility (Vince-Prue 1975; see also Section 8.4). Other evidence from action spectra emphasises the importance of red wavelengths of light for SDPs in contrast to the response to far-red for LDPs.

(b) Photoperiodic timekeeping

Accurate measurement of daylength for control of flowering requires a 'photo' response via a photoreceptor, and a measure of 'period' generally involving a circadian, rhythmic, timer. Circadian, meaning 'about a day', refers to the natural period of these rhythms often being not exactly 24 h. In the absence of external stimuli, most rhythms manifest as free-running circadian cycles. However, the timing of dawn and/or dusk entrain the rhythm to synchronise with exact 24 h cycles and hence provide an accurate daily clock used by both SDPs and LDPs. The currently favoured explanation of photoperiodic timekeeping involves rhythmic biochemical processes.

In addition, phytochrome is clearly involved (Figure 8.27), but may not act as an instantaneous on/off switch with respect to the light/dark cycle. Phytochrome is rapidly activated in light but on return to darkness there can be a slow (~ 0.5 to 4 h) delay in disappearance of active phytochrome (the Pfr form) as it is degraded or decays back to the inactive Pr form. The consequence may be an offset between when it is actually dark and when the plant perceives it is dark. In the 1950s, Borthwick and Hendricks proposed that this natural offset, acting like an hourglass, accounted for photoperiodic time measurement in flowering (Hendricks 1960). Nowadays, the hourglass theory is often dismissed, especially as it would be limited to measuring dark periods only up to 4 h. However, it does provide a rational explanation of flowering of SDPs exposed to an extended long dark period and may well be a necessary component of photoperiodic timekeeping but perhaps not the limiting factor. There may also be an essential stabilisation period after Pfr decay during which other forms of timing may occur.

Although daily light/dark cycles set the phase and entrain 24 h rhythms, this does not explain photoperiodic control of flowering. For example, there are distinct phase settings of leaf movement rhythms for the SDP *Pharbitis nil* when in long or short days, but flowering is stimulated only by short days. In 1936, Bünning deduced that there is a second, additional, light response allowing or preventing expression of the rhythm (see Bünning 1960; Lumsden 1991). The phase of the rhythm imposes or determines sensitivity of flowering to this second light input. The consequence is that, depending on daylength, light may or may not be synchronised with the dark-requiring part of the rhythm (Figure 8.29) and so flowering is either prevented or allowed.

Other rhythms have been revealed at the genetic and molecular levels. For example, *Arabidopsis* plants transformed with a luciferase gene (see Chapter 10) for bioluminescence coupled to the promoter sequence for a clock-regulated plant gene gave a simple, visually assayed, indicator rhythm which was then used to screen for period length mutants (Millar *et al.* 1995c). None of the mutants influenced flowering response, so it appears that there may be several independent clocks operating.

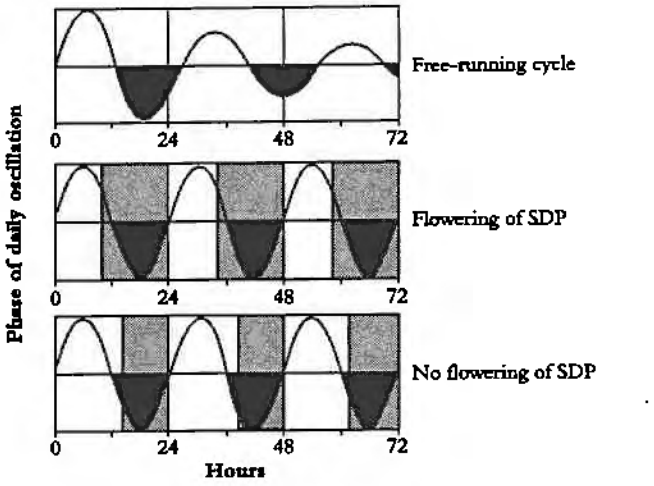


Figure 8.29 Daily light/dark cycles (empty/hatched areas) phase and entrain a free-running circadian (from *circa-diem*, meaning 'about a day') oscillation to an exact 24 h cycle. It is proposed that one-half of the cycle tolerates light with the other half (darkened portion) being intolerant. Thus, for the SDP, flowering is only permitted with long dark periods. However, the duration of light and darkness are both crucial components of time measurement
(Based on Bünning 1960)

(c) Floral stimuli and inhibitors

The diverse environmental influences on flowering make it unlikely that plants possess a simple, unique regulatory signalling system. At least for photoperiod responses, grafting experiments indicate the presence both of transmissible promoters and inhibitors. However, isolation of florigenic chemicals from induced plants (Table 8.10) remains at a preliminary stage. We are still uncertain whether the floral stimulus (or inhibitor) is a single compound, a complex of compounds, whether it is photoperiod class specific, species specific or more universal.

Grafting experiments have confirmed that leaves produce photoperiodic stimuli that are transmitted to the shoot apex, as discussed earlier for *Perilla* (Figure 8.26). For several long-day and short-day species, pre-induced, grafted leaves or leafy shoots cause flowering of vegetative recipient plants held in

Table 8.10 Effect of plant extracts on flowering response of seedlings of the SDP Chenopodium rubrum *held in long days. Extracts were of tobacco leaves from plants in* inductive *short days or in non-inductive long days*

	Flowering (%)		
	Vegetative	Pre-floral	floral
Short-day leaf extract (inductive treatment)	0	55	45
Long-day leaf extract (non-inductive treatment)	100	0	0
No-extract control			

(From Chailakhyan *et al.* 1989)

Graft donor / Graft receptor	Trapezond DNP / Trapezond DNP	Maryland Mammoth SDP / Trapezond DNP	*N. sylvestris* LDP / Trapezond DNP
Daylength		**Days to flower**	
Long day	49	> 48	23
Short day	49	32	∞

Figure 8.30 Evidence for presence of graft-transmissible inhibitors of floral induction. Flowering of the tobacco line Trapezond is indifferent to daylength (day-neutral plant, DNP) but Trapezond receptor shoots (↗) show delayed flowering if grafted with short-day (Maryland Mammoth, ●) or long-day (*Nicotiana sylvestris*, ▲) tobaccos held in unfavourable photoperiods. Conversely, favourable photoperiods lead to transmission of a floral promoter
(Based on Lang *et al.* 1977)

non-inductive conditions (see Lang 1965 and Bernier *et al.* 1981). Intriguingly, grafted leaves from day-neutral species may even be effective donors to LDPs or SDPs held in non-inductive photoperiods. In a few cases, such as *Sedum spectabile* (LDP) and *Kalanchoe blossfeldiana* (SDP), interspecies grafts have also been successful. This tells us that, despite photoperiodic differences, there may be common stimuli or common perception by the apex of different stimuli.

Many unsuccessful, frustrating attempts to extract and identify flowering stimuli have led florigens sometimes to be called hypothetical, non-existent or the holy grail of plant physiology. In addition to the tobacco extract example in Table 8.10, some positive results have also been reported for the SDP *Pharbitis nil* (Ishioka *et al.* 1991). In both studies, there was activity only in extracts from induced plants. Importantly, there was no activity for extracts of non-induced long-day leaves or their phloem exudates. We predict from experiments measuring speed of transmission that the signal moves in the phloem but no florigen has been chemically identified. The identity of inhibitory compounds is a further mystery. The main evidence for floral inhibitors comes again from grafting studies, for example in day-neutral tobacco. When grafted with a LDP tobacco, *Nicotiana sylvestris* (Figure 8.30), the day-neutral line flowers late if the graft partner is in non-inductive conditions; we deduce that it is producing an inhibitor that can pass across the graft union. The converse experiment with the long-day partner in inductive days led to early flowering of the day-neutral plant, so there is also a transmitted promoter (Figure 8.30). However, 'Maryland Mammoth', a short-day tobacco, lacks the graft-transmissible inhibitor, indicating how difficult it is to unravel the complexities of signalling.

(d) Hormonal involvement

One reason for considering a role for plant hormones in the regulation of flowering is the frequent reports that their application dramatically alters flowering. However, correlations with altered endogenous hormone levels are not always evident, for example in the case of ABA content during floral induction in *Lolium*. By contrast, gibberellin application can cause flowering particularly of rosette plants. It may replace a need for vernalisation or long days in control of bolting and flowering (Lang 1965) and, as we will see later, endogenous gibberellin content may also increase following environmental changes that lead to flowering.

Some commercial uses of hormones have followed. For example, ethylene synchronises flowering and fruiting of bromeliads and is used worldwide for pineapple production. Conversely, inhibition of flowering of sugar cane by ethylene is practised in Hawaii where yield is greater if flowers do not develop (Moore and Osgood 1986).

With some ornamental species such as *Spathiphyllum*, most commercial growers use gibberellin because one application halves the time to flowering from six to three months. This early flowering is probably not related to juvenility, which is sometimes extended by applied gibberellin as in ivy (*Hedera* sp.) and shortened in *Eucalyptus nitens* when gibberellin levels are lowered. After treatment with paclobutrazol, which blocks gibberellin biosynthesis, grafted seedlings flower massively and three to five years earlier than normal (see earlier comment on juvenility and Moncur and Hasan 1994). Yet we find there are no generalisations. For conifers, high gibberellin level may overcome juvenility and applied gibberellins, in combination with harsh cultural conditions, allow flowering at one to two years rather than after 10 to 20 years (see Pharis and King 1985). For some non-rosette species, long days and/or vernalisation can lead to rapid increases in gibberellin content (Metzger 1995) and inhibition of gibberellin biosynthesis may also block or delay flowering, which further suggests a link between gibberellins and normal reproductive responses. In species with no juvenile phase, gibberellins may replace the need for long days or vernalisation. For example, in the LDP *Arabidopsis*, a dwarf mutant (*ga1-3*) which is blocked in gibberellin biosynthesis, flowers later than its wild type. In short days, some of these mutant plants may never flower unless treated with gibberellin (Table 8.11). On the other hand, vernalisation fails to stimulate flowering. Evidence against a role for gibberellins comes from the normal flowering of dwarf genotypes of many species (e.g. pea, corn, wheat, rice) which are blocked in gibberellin biosynthesis or in capacity to respond to gibberellin (e.g. pea, corn, wheat, rice)(see summary in(Reid and Howell 1995).

Gibberellins can instead be inhibitory, especially for some perennials, including *Fuchsia*, *Bougainvillea*, mango and citrus, and also for species such as strawberry. Other gibberellins are known which can stimulate flowering without affecting

Table 8.11 Days to flower for Arabidopsis thaliana *wild type and gibberellin-deficient or gibberellin-insensitive mutants held in short days (SD) (8 h photoperiod). Gibberellin (GA_3) treated plants were sprayed weekly beginning 17 d after planting*

	Days to flower	
	SD Control	SD + GA_3
Wild type	47	32
gal-3 mutant (deficient)	>117	44
gai mutant (insensitive)	75	75

(From Wilson *et al.* 1992)

growth. A more extreme response is seen from some novel synthetic gibberellins which can even act as growth retardants while still retaining ability to promote flowering (Evans *et al.* 1994b, c).

Complex relationships also exist between cytokinins and flowering. In the LDP *Sinapis*, endogenous cytokinin levels increase up to three-fold in long days. Applied cytokinin, however, induces only a partial flowering response (Bernier *et al.* 1993). There can also be indirect effects as found in *Pharbitis nil* where cytokinins can alter assimilate distribution to give either inhibition or promotion of flowering (Ogawa and King 1979).

We know much less about genetic and molecular events around the time of floral induction. Beginning with a late flowering mutant in *Arabidopsis*, a gene, *CONSTANS*, has been identified whose expression is upregulated by long days (Putterill *et al.* 1995) and which may be one step in the sequence to florigens. Manipulation of phytochrome genes influencing flowering has also provided information on photoperiodic processes in leaves. In the future, we can expect to find links to timekeeping genes which influence endogenous rhythms. Analogous genes have been isolated from other organisms including *Neurospora* and *Drosophila*.

8.4 Photoreceptors and light cues

Light is the energy source that drives plant life so it is no surprise that plants generally maximise the interception of solar radiation. These strategies range from the complexities of chloroplast ultrastructure to tree architecture. Energy for photosynthesis is harvested by chlorophyll and accessory pigments (Section 2.3), but plants also possess other light-absorbing molecules that have evolved to sense light intensity, light duration, light direction and spectral composition. These *photoreceptors* are coupled to many developmental processes. For example, the developmental strategy of a seed on the soil surface with immediate access to sunlight is quite different from one buried under several centimetres of soil. The initial growth phase of the latter needs to be rapid and upwards and to consume as little of the seed's resources as possible. That is why seeds germinated in the dark have spindly stems, aren't green (because there is no possible photosynthesis) and don't expand their leaves (because this is unnecessary and they will cause friction as the shoot grows through the soil). When the shoot tip does reach light, there is a complete reassignment of priorities resulting in assembly of functional chloroplasts, expansion of leaves and reduction in stem elongation. These processes are coordinated by two main classes of photoreceptor: *phytochromes* and *blue-light receptors* (also known as *cryptochromes*). Here, we consider briefly the operation of these light sensors at the molecular and physiological levels.

(a) Phytochromes — multifunctional light sensors

Early studies of plant developmental responses to light were some of the most fascinating and elegant, and led to the conclusion that not only was light quantity important but different wavelengths caused different reactions (Borthwick *et al.* 1954). In particular, several processes (e.g. seed germination, floral induction) responded to red (R; around 660 nm) and far-red (FR; around 730 nm) wavelengths in quite opposite ways. This turned out to be a manifestation of the operation of one set of morphogenetic pigments, the phytochromes. We now know, from isolation of phytochrome in a test-tube, and later discovery of several phytochrome genes, that phytochromes are complex molecules consisting of a protein linked to a chromophore (Figure 8.31). Photon absorption by the latter causes a conformational change which alters the *absorption spectrum* (Figure 8.32a). In most types of phytochrome, these changes can occur repeatedly, a phenomenon known as photoreversibility. The two states are termed the Pr form and Pfr form, because of their optimum absorbances in the R and FR regions, respectively. Note that Pfr absorbs to some extent in the red region, which means that irradiation with pure red (660 nm) will lead to absorption by both forms and so interconversion will continue indefinitely. Eventually, however, a

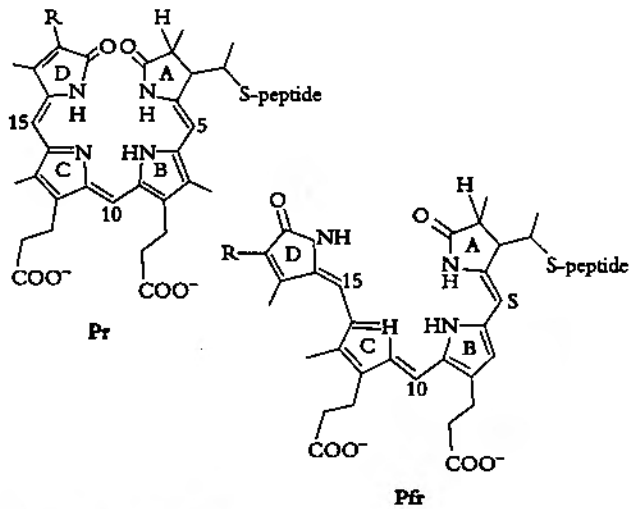


Figure 8.31 Phytochromes consist of a chromophore a protein linked through the sulphur atom of a cysteine amino acid residue to a protein ('peptide' on diagram) a chromophore. Absorption of light causes a reversible conformational change in the chromophore (a *cis–trans* isomerisation centred on carbon 15) which alters the absorption spectrum. The two forms are referred to as Pr (left) and Pfr (right). Most phytochrome responses are activated when molecules are in the Pfr form
(Reproduced, with permission, from Salisbury and Ross 1992)

stable state is reached, called the photostationary equilibrium, in this case with about 15% of molecules as Pr and 85% as Pfr. Because Pr absorbs very little far-red, pure far-red leads to about 97% Pr and 3% Pfr. Normally, of course, plants are exposed to sunlight which contains red *and* far-red wavelengths (Table 8.12). The link to the physiological responses — from experiments done under lots of different wavelengths leading to graphs known as *action spectra* (Figure 8.32b) — is now a lot easier to understand. Conversion of Pr to Pfr by red light is the basis of red-promoted processes. Although the classic photoreversible phytochrome responses show that Pfr is the active form, there is also evidence that Pr is important, for example in maintaining shoot gravitropism in the dark (Liscum and Hangarter 1993). Surprisingly, phytochrome is also present in roots, with Pr having a role in regulating elongation growth.

Table 8.12 Typical light conditions found in nature. Plants use a range of different photoreceptors to sense the light quantity, here measured as photosynthetically active radiation (PAR). Spectral composition in the red/far-red region is detected by phytochromes

Situation	PAR (μmol m^{-2} s^{-1})	Incident R:FR light ratio (660/730 nm)
Mid-day sunlight	2000	1.19
Forest canopy	20	0.13
Twilight	1	0.96
Under 10 mm soil	0.01	0.88
Moonlight	0.0001	0.94

(From Smith and Whitelam 1990 and Smith 1982)

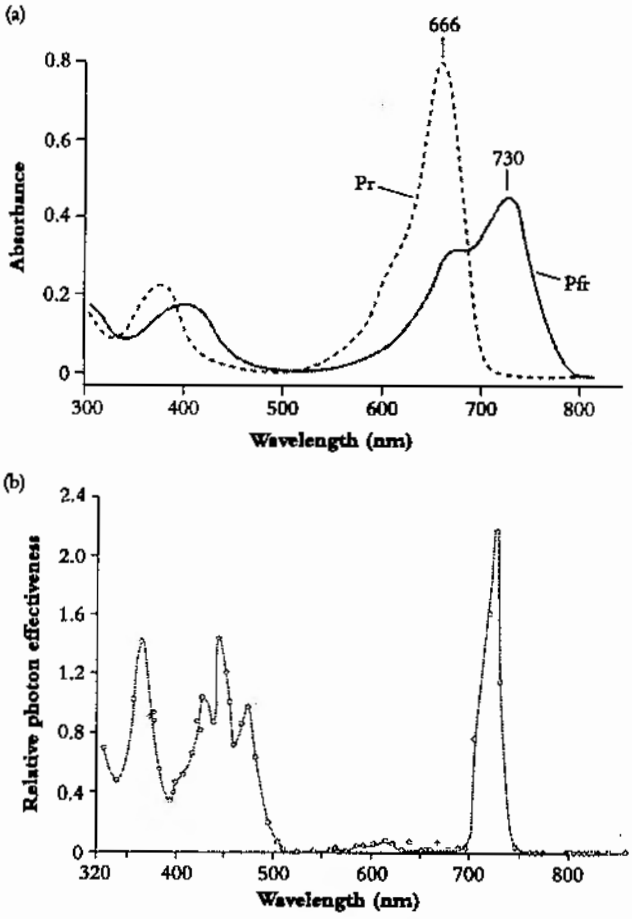


Figure 8.32 Phytochrome can be characterised chemically by its light absorption spectrum, and biologically by its action spectrum. (a) Absorption spectra of Pr and Pfr. Although Pr and Pfr both absorb in the blue and ultraviolet regions, their biological importance relates mainly to the difference in the red and far-red regions. Conventionally, Pr and Pfr maximum absorbances are taken as 660 nm and 730 nm, respectively. (b) Action spectrum of inhibition of hypocotyl elongation in dark-grown lettuce seedlings. The maximum effect is at 720 nm, in the far-red zone. The effectiveness of wavelengths <500 nm is due to blue-light receptors, discussed later in the text

(From Vierstra and Quail 1983 and Hartmann 1967)

(b) *PHY* genes and classes of phytochrome operation

We now know that there are at least two main phytochrome response classes (Type I and Type II), and probably more than one gene coding for each. For example, there are five genes (*PHYA* to *PHYE*) in the model plant *Arabidopsis*, and seven in tomato (Smith 1995). In the past, sometimes confusing terminology has reflected our incomplete understanding of the differences between the various forms and genes. What is clear is that Type I responses relate to phytochrome A (phyA) which is the most abundant in dark-grown seedlings, up to 99% of the total phytochrome. Type I is photoreversible, but in the Pfr form phyA is also very unstable with a half-life of about 1 h, so that after exposure of a plant to a few hours of light, most

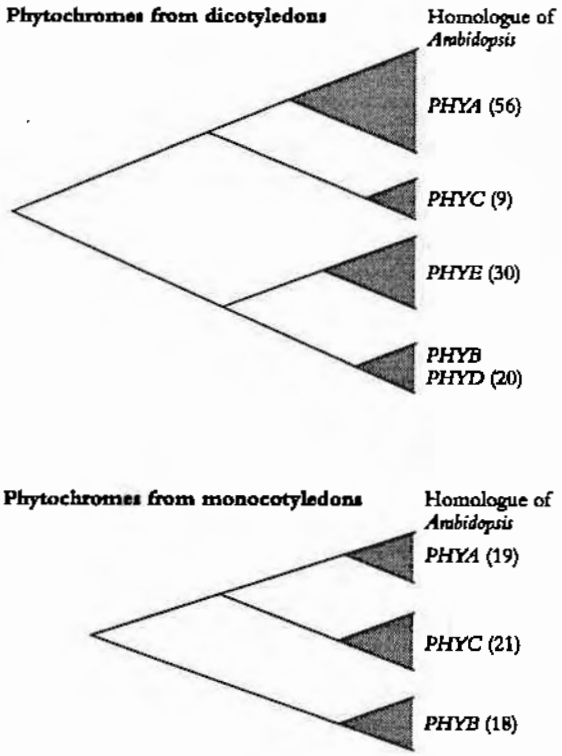


Figure 8.33 The phytochrome gene family has several members with differing degrees of sequence homology (i.e. molecular similarity), indicated by the branch lengths on these diagrams. Of the Type II phytochromes (*PHYB* to *PHYE*), which are a grouping based on physiological response, *PHYC* appears to be genetically distinct and is more closely related to *PHYA*. The data are assembled from gene database information for 172 species of flowering plants. Numbers in parentheses represent the number of nucleotide sequences found in each *PHY* class

(Reproduced, with permission, from Mathews and Sharrock 1997)

of the phyA has been degraded (Clough and Vierstra 1997). Sometimes this is called 'light-labile' phytochrome, but degradation of Type I Pfr continues unabated in the dark. Type II (various versions coded by genes *PHYB* to *PHYE*) is present at only a few per cent of the original Type I concentration. Pfr Type II has a much longer half-life, in dark and in light. Type II phytochromes are responsible for classic photoreversible (R-promoted, FR-inhibited) processes and for sensing spectral R:FR ratios. By surveying DNA sequence homology of phytochrome genes across many species, a generic model has been developed of how closely related the various forms are, from which can be deduced their probable evolutionary history (Figure 8.33).

(c) Phytochromes operation and light quantity

The quantity of light required to initiate phytochrome responses varies enormously. At one end of the range, very low fluence responses (VLFR) are amazingly sensitive, requiring around 10^{-8} moles of quanta m^{-2}, equivalent to 2 min of moonlight or a mere 0.5 ms of full sunlight (Smith and Whitelam 1990). The VLFR class is mediated by phyA and is not reversible by far-red light because at the light intensities involved far-red-induced reversion of Pfr to Pr is insignificant compared with other mechanisms of Pfr degradation. The high concentration of phyA in dark-grown tissues is probably an adaptation for maximised sensitivity to minuscule amounts of light. The VLFR mode operates exclusively in tissues in darkness, especially deep-buried seeds that may germinate in response to light penetrating through the soil, or a seedling shoot detecting its first few photons, allowing early warning of arrival at the soil surface and initiating conversion to de-etiolated development.

Low fluence responses (LFR) also operate with very little light and saturate after the equivalent of 1 s of full sunlight. Unlike VLFR, this class operates via Type II phytochromes and is typified by the classic R–FR photoreversible response, and by perception of spectral quality (R:FR ratio) involved in growth adjustments under leaf canopies. It is interesting to note that the latter is manifested as an increase in shoot extension rate, whereas at VLFR intensities, the same wavelengths can cause decreased elongation.

High-irradiance response (HIR) is a slightly misleading term because, although requiring more sustained light than LFR, these responses still operate at only a few per cent of full sunlight. HIR covers several different types of response, but sometimes is rather unhelpfully used to include blue-light responses (see below) that do not involve phytochrome at all. Both red and far-red can initiate HIR through Type II and Type I phytochromes respectively. The latter is probably part of the daylength perception system in LDPs. Many other far-red-induced HIR disappear soon after plants are exposed to light, presumably because most of the phyA has been degraded.

(d) *phy* mutants

Sorting out which phytochrome type is associated with each physiological response has been aided greatly by phytochrome mutants, mostly in *Arabidopsis*, but also in pea, tomato and sorghum. Some of the mutants have a defective chromophore, others have lesions in the protein part of the molecule. For example, *phyB* mutants exhibit changes in germination, elongation growth, flowering time and chlorophyll accumulation. This suggests that each phytochrome has multiple functions. Some of these processes are also altered in *phyA* mutants, but often in subtly different ways. We can tentatively conclude that phytochromes interact to orchestrate many aspects of plant development. Smith (1995) has attempted to put all these functions into an ecological context, and has assigned each to a particular class of response (Figure 8.34).

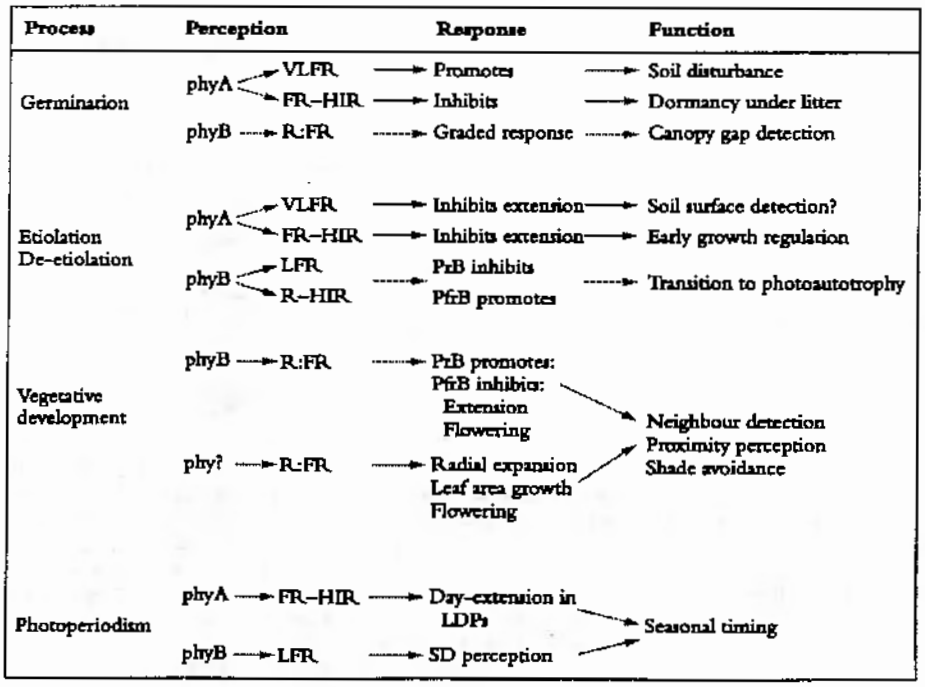


Figure 8.34 Multiple phytochrome sensing systems enable plants to adjust development under a wide range of ecologically important light environments. VLFR = very low fluence response, LFR = low fluence response, HIR = high irradiance response
(Reproduced, with permission, from Smith 1995)

(e) Blue-light receptors and responses

Although Julius von Sachs in the 1860s discovered that blue light caused phototropism, photomorphogenesis under blue-light control has long been the poor cousin of studies on phytochrome. However, since the 1980s, enormous progress has been made, leading to characterisation of a blue-light receptor, sometimes called cryptochrome, that is quite unrelated to phytochrome. Responses to blue light require relatively high light intensities, but can occur extremely fast — electrical potentials across the plasma membrane can alter within 15 s, and cucumber seedling growth can be reduced within 30 s of transferring from dark to blue light (Figure 8.35). Speeds of this order tell us that some blue-light responses are initiated without any need for a change in gene expression. Although blue light is also the prime causative agent in phototropism (Figure 8.36 and see Section 8.2.5), this differential growth response has a much longer lag time, usually around 30 min, than in the straight growth inhibition mentioned above. As with phytochrome, we now know that there are multiple forms and genes for the blue-light receptor (Cashmore 1997), each comprising a protein and two chromophores, one of which is flavin adenine dinucleotide (FAD) and the other possibly a pterin. However, it is not clear which of these functions as the receptor for phototropism. Briggs and Liscum (1997) concluded from studies with the *hy4* (hypocotyl length) and *nph* (non-phototropic hypocotyl) mutants of *Arabodopsis* that elongation growth and phototropism are under genetically independent control.

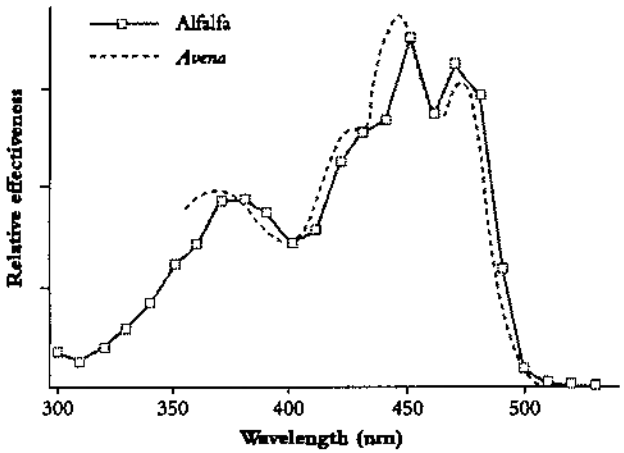


Figure 8.35 A blue-light receptor is responsible for phototropism. Action spectra for monocotyledons (*Avena*, oat) and dicotyledons (alfalfa) are very similar, and suggest that a flavin is part of the chromophore
(Reproduced, with permission, from Baskin and Iino 1987)

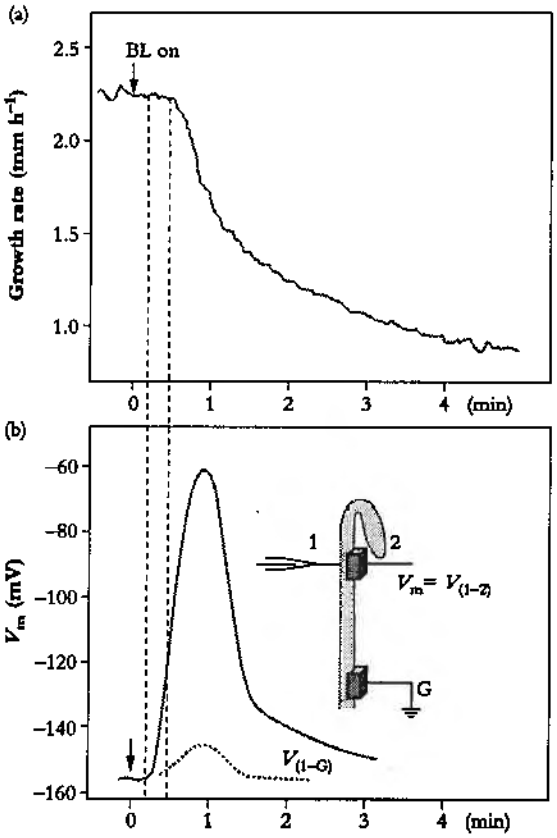


Figure 8.36 Responses to blue light (BL) can be very rapid, often much faster than those mediated by phytochrome. Here, growth rate (a) decreases after 30 s and plasma membrane electric potential (b) changes even sooner, within 15 s, when blue light (10 μmol m^{-2} s^{-1}) is applied to hypocotyls of dark-grown cucumber seedlings
(Based on Spalding and Cosgrove 1988)

(f) Conclusion

Multiple phytochrome genes and response classes, together with blue-light receptors, confer on plants a remarkable repertoire of light-sensing systems that operate through all stages of the life cycle and are effective across every light condition present in nature (Table 8.12). Starting with triggering or inhibiting germination, and the conversion from etiolated seedling growth to development of photosynthetic apparatus, photoreceptors assist plants to optimise their development, and phytochrome later becomes involved in photoperiod perception for flowering (see Section 8.3.2). Coping with growth under forest canopies, attempts to avoid shade and to perceive neighbouring plants — these all relate to sensing of direct sunlight and of light transmitted or reflected by other vegetation.

Further reading

Bernier, G., Kinet, J.M. and Sachs, R.M. (1981). *The Physiology of Flowering*, Vol. 1, CRC Press: Boca Raton, Florida.

Bewley, J.D. and Black, M. (1982). *Physiology and Biochemistry of Seeds*, Springer-Verlag: Berlin.

Bewley, J.D. and Black, M. (1994). *Seeds. Physiology of Development and Germination*, Plenum Press: New York.

Cashmore, A.R. (1997). 'The cryptochrome family of photoreceptors', *Plant Cell and Environment*, **20**, 764–767.

Dennis, F.G. (1994). 'Dormancy — what we know (and don't know)' *HortScience*, **29**, 1249–1255.

Evans, L.T. (1993). *Crop Evolution, Adaptation and Yield*, Cambridge University Press: Cambridge.

Firn, R.D. and Digby, J. (1980). 'The establishment of tropic curvatures in plants', *Annual Review of Plant Physiology*, **31**, 131–148.

Halstead, T.W. and Dutcher, F.R. (1987). 'Plants in space', *Annual Review of Plant Physiology*, **38**, 317–345.

Hart, J.W. (1988). *Light and Plant Growth. Topics in Plant Physiology*, eds M. Black and J. Chapman, Unwin Hyman: London.

Hart, J.W. (1988). *Plant Tropisms and Other Growth Movements*, Unwin Hyman: London.

Lang, G.A. (ed.) (1996). *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*, CAB International: Oxford.

Langkamp, P. (1987). *Germination of Australian Native Plant Seed*, Inkata: Melbourne.

Mott, J.J. and Groves, R.H. (1981). 'Germination strategies', in *The Biology of Australian Plants*, Eds J.S. Pate and A.J. McComb, 307–341, University of Western Australia Press: Nedlands, WA.

Reid, J.B. and Howell, S.H. (1995). 'The functioning of hormones in plant growth and development', in *Plant Hormones*, ed. P.J. Davies, 448–485, Kluwer Academic Publishers: The Netherlands.

Reid, J.B. and Murfet, I.C. (1975). 'Flowering in *Pisum*: the sites and possible mechanisms of the vernalisation response', *Journal of Experimental Botany*, **26**, 860–867.

Saure, M.C. (1985). 'Dormancy release in deciduous fruit trees', *Horticultural Reviews*, **7**, 239–300.

Simons, P. (1992). *The Action Plant. Movement and Nervous Behaviour in Plants*, Blackwell: Oxford.

Salisbury, F.B. and Ross, C.W. (1992). *Plant Physiology*, 4th edn, Wadsworth Publishing: Belmont, California.

Smith, H. (1982). 'Light quality, photoperception, and plant strategy', *Annual Review of Plant Physiology*, **33**, 481–518.

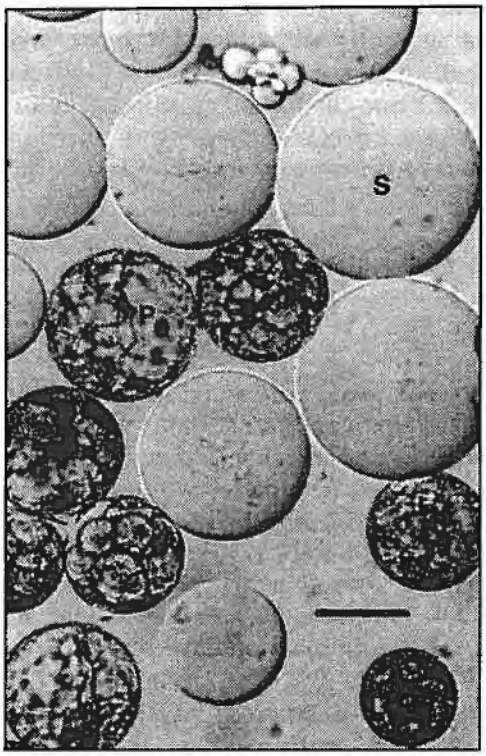
Smith, H. (1995). 'Physiological and ecological function within the phytochrome family', *Annual Review of Plant Physiology and Plant Molecular Biology*, **46**, 289–315.

Trewavas, A.J. (1982). 'Growth substance sensitivity: the limiting factor in plant development', *Physiologia Plantarum*, **55**, 60–72.

Trewavas, A.J. and others (1992). 'Discussion forum: What remains of the Cholodny–Went hypothesis', *Plant Cell and Environment*, **15**, 757–794.

Vince-Prue, D. (1975). *Photoperiodism in Plants*, McGraw Hill: London.

9 *Plant hormones: chemical signalling in plant development*



Consider... a plant not as a packaged collective of independent processes but as a highly interactive network of perception, control and feedback. Every plant has a genetic blueprint that specifies its whole range of morphology and physiology, but the individual is shaped, sometimes literally, by the environment it experiences. Integration of development and adjustment to the external environment are achieved through multiple coordinating signals throughout the plant.

Perception of gibberellin in germinating cereals. Protoplasts (P) isolated from aleurone cells of wild oat (Avena fatua) were incubated with Sepharose beads (S) to which gibberellin molecules had been covalently attached. The gibberellin therefore could not enter the cells, but was still able to induce production of α-amylase enzyme. This means that perception of gibberellin probably occurs via and outward-facing receptor in the plasma membrane. Scale bar = 60 μm (? see Colour Plate xx) *(Reproduced, with permission, from Hooley et al. (1991)*

Chapter outline

Introduction: the need for communication

In the previous chapter we introduced some of the complexity and subtlety of the functioning of plants in diverse, variable and unpredictable environments. Their sessile nature makes it a necessity for plants, if they are to succeed as individuals and populations through many generations, to have the resourcefulness to cope with and adjust to environmental change, especially at the extremes. Remember that plant physiologists are probably the only people on earth who routinely grow plants under constant environments in growth chambers! In this chapter we examine some of the internal mechanisms that plants use to coordinate development. Let us start by considering the concept of a plant not as a packaged collective of independent processes but as a highly interactive network of perception, control and feedback. A plant has a genetic blueprint that specifies its normal morphology and physiology throughout the whole life cycle, but every individual is also shaped, sometimes literally, by the environment it experiences. Think of the bent-over shape of trees growing on coasts with a prevailing on-shore wind, or the ability of pasture plants to recover repeatedly from grazing of their shoot tips.

We might first ask whether plants really need internal communication. The answer lies with multicellularity. With multicellularity comes almost invariably differentiation. Differentiation is in effect specialisation, which can also be thought of as division of labour. The particular physiological and developmental facets of an organ, tissue or cell type (say, a leaf, a phloem bundle and a guard cell, respectively) make it more efficient at carrying out its set of functions. But with specialisation comes a dependency on the rest of the organism, and a need for coordination between its component tissues. Some of the control is attributable to *resource limitations*: water, light, CO_2 and inorganic nutrients in the environment; water, carbon, nitrogen and minetal fluxes inside the plant. Many of these factors are discussed in Part IV of this book. Depending on quantities and types of resources available, and their mobility in the plant, there are undeniable limits placed on the scope of development. A shoot system can develop only as rapidly as the root mass can supply water and minerals for the shoot structure; a root can grow only if fed with fixed carbon, normally from the shoot. These ubiquitous molecules function as integral parts of cell structures and core metabolism. What we find in addition is another layer of control: *information-rich mobile molecules* that serve as an *integrating communication system* throughout the plant.

Animals have a central nervous system and a suite of specific hormones each with highly defined functions. Plants lack the former, but do possess a quite different set of chemical signals called *plant hormones* (Table 9.1). Plant hormones are sometimes called 'plant growth substances' or 'plant growth regulators' partly to distance them from mammalian concepts of hormone action. However, these alternative terms undervalue the repertoire of functions of plant hormones: they affect so many processes other than just growth, so we continue to refer to endogenous regulatory substances as 'plant hormones'. We also talk later (see Section 9.3) about plant growth regulators as a broader group of active substances applied to plants which includes more than just plant hormones.

Table 9.1 A comparison of major features of plant and animals and their regulatory systems

Plants	Animals
Sessile	Motile
Autotrophic	Heterotrophic
No nervous system	Nervous system
Plastic development throughout lifespan	Adult development determinate
Mostly passive mass-flow systems	Active circulatory systems
Hormones produced in many locations	Hormone production in restricted cell types
Hormones multifunctional	Hormones more specific in function
Development highly sensitive to environmental influences	Development relatively unaffected by environment

Before describing specific plant hormone functions, we need to consider how hormone signals might operate effectively. In any signalling system there is a source and a target, and in between a mode of transmission — in radio parlance, the transmitter and receiver with signals travelling as electromagnetic airwaves. In animals, the conventional system is a source gland, mass-flow transport (e.g. blood circulation) and a target tissue. Plants are harder to diagnose, but we can make the following generalisations:

- Each hormone can be synthesised in more than one location in a plant. Indeed, all living cells may produce all hormones, but some generate larger quantities and others almost undetectable amounts.
- Each hormone has many functions, at least by deductions from experiments with applied hormones and from phenotypes of hormone-deficient and hormone-insensitive mutants.
- Plant hormones are small molecules and are mobile, both over short (diffusive) and long (mass-flow) distances.

- Many cell types respond to each hormone class.
- Some hormone functions occur in the same cells or tissue in which they are synthesised.

From this, we conclude that a plant's hormones are indeed quite different from those in animals (Table 9.1). There are relatively few classes but each is multifunctional, they are not synthesised in glands, they move in several channels and affect several tissues in a multitude of ways. A recipe for crossed wires and confusing ambiguity of signals? Perhaps, but as we introduce the major hormones, an overall picture of plant communications will emerge. We now examine signal sources and signal mobility, and then consider how signals are perceived and translated into altered physiology and development.

9.1 The basis of chemical control of plant development

9.1 Signal sources: which tissues make hormones? How are hormones synthesised?

Traditionally, five major hormone classes are described: auxins, cytokinins, gibberellins, abscisic acid and ethylene. Other active compounds have been known for years, and there are newcomers with increasingly strong claims for inclusion. These include brassinosteroids and jasmonic acid in particular, but also polyamines, salicylic acid and oligosaccharides; for details of these, some informative review articles are listed at the end of the chapter.

Most hormones have certain biochemical trends in common: small molecules synthesised from ubiquitous precursors (amino acids, mevalonic acid, nucleotides) sometimes via multi-step pathways, then deactivated by oxidation or *conjugation* (linking to other small molecules such as glucose and amino acids). Some knowledge of hormone biochemistry will be invaluable in Section 9.3 where we describe genetic and chemical approaches to manipulating plant development through modified hormone biosynthesis and degradation. Further information on hormone biochemistry can be found in many recent reviews and texts (e.g. Davies 1995).

Auxins

Auxin in its most common natural form indole-3-acetic acid (IAA; Figure 9.1a), was the first plant hormone to be isolated, and was long thought to be derived exclusively from the amino acid tryptophan. Plants and certain plant pathogenic bacteria synthesise IAA, although the genes, enzymes and reaction intermediates differ between prokaryote and eukaryote. From data on tryptophan-deficient mutants, it now appears that indole may be an alternative starting point for IAA synthesis in some plants (Wright *et al.* 1991; Normanly *et al.* 1995). This advance illustrates our incomplete knowledge of even elementary plant hormone biochemistry. Active growing tissues, especially shoot tips and young leaves, synthesise auxins, as do developing fruits and seeds. Roots appear to produce much less auxin, but auxin has vital *functions* in lateral root development. Plants and bacteria can deactivate auxins by irreversible oxidation involving enzymes such as IAA oxidase, or by covalently linking (conjugating) them to other small molecules: sugars, cyclitols, amino acids. Some conjugates (e.g. IAA-aspartate) act as inactive auxin stores, regenerating active auxin when the link is hydrolysed.

Cytokinins

In many ways, cytokinins are opposites of auxins, being synthesised in roots but with most dramatic effects on shoot development. However, shoot tissues can also produce cytokinins, as can developing seeds. A classic example of the latter is coconut milk, the copious liquid endosperm from coconut seed, which is still a popular cytokinin source in plant tissue culture media. Cytokinins were originally named from their ability to promote cell division, but they also function in initiation of new shoot structures, dormancy release and retardation of senescence. Cytokinins are derivatives of adenine, one of the purine bases found in all DNA and RNA. Indeed, cytokinins were originally thought to be products of transfer RNA (tRNA) breakdown. However, based on cytokinin and tRNA composition of pea roots and turnover rates in maize, it was calculated that there were insufficient cytokinin nucleotides in tRNA to account for total cytokinin production (Short and Torrey 1972; Klemen and Klämbt 1974). Instead, a *de novo* pathway using free adenine nucleotides as substrate appears to predominate. There are also many cytokinin types each with subtle differences in structure. The four main classes of natural cytokinin each have a different five-carbon side-chain attached to the N_6 position (Figure 9.1b). The two classes found in tRNA (*cis*-zeatin and isopentenyladenine) are less biologically active than the major free cytokinin classes, *trans*-zeatin and dihydrozeatin. Discovery of a *cis-trans* isomerase that interconverts the two zeatin forms has re-opened the biosynthesis debate, because active *trans*-zeatin may be made from RNA-derived *cis*-zeatin (Bassil *et al.* 1993). The first enzyme in the *de novo* pathway, isopentenyl transferase (IPT), is well known in bacteria but has yet to be characterised fully from plant tissues. A novel suggestion is that all cytokinins in plants are actually synthesised by bacteria present on and in plant tissues (Holland 1997). If true, this could account for the failure to find plant biosynthetic enzymes, and for the lack of cytokinin biosynthesis mutants. Each cytokinin class exists as base, riboside and nucleotide forms, many of which can readily be metabolically interconverted. This has made it difficult to decide which forms are biologically active in their own right, and which achieve activity only after conversion. As with auxins, inactivation results from conversion to glucosyl or

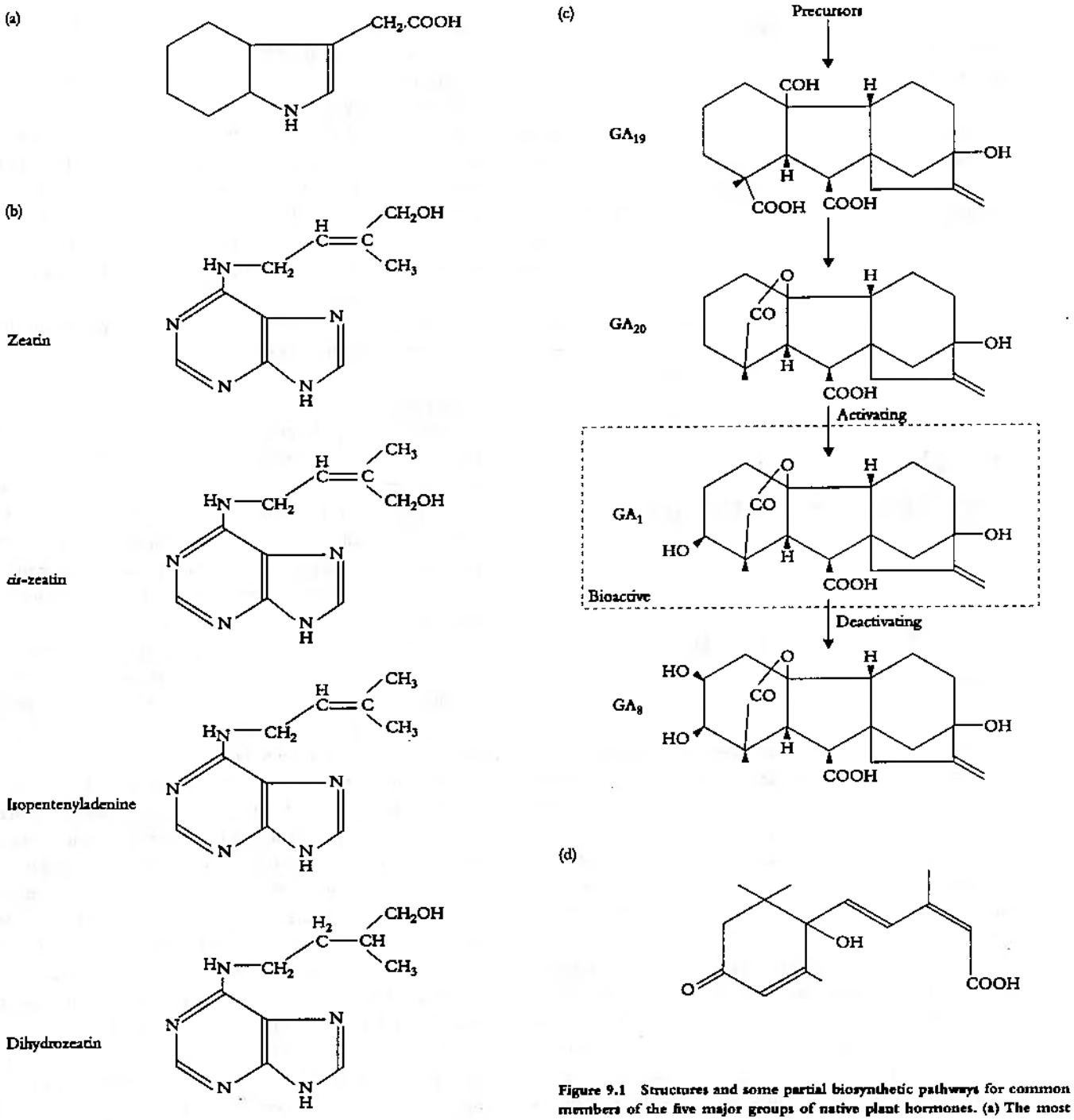


Figure 9.1 Structures and some partial biosynthetic pathways for common members of the five major groups of native plant hormones. (a) The most common natural auxin, indole-3-acetic acid (IAA). (b) Four classes of natural cytokinin: zeatin and its *cis* isomer, dihydrozeatin, isopentenyladenine. (c) Late stages of gibberellin biosynthesis pathway, showing some key points of genetic and environmental control of amounts of bioactive GA_1.

(i) The $GA_{19} \rightarrow GA_{20}$ step is under photoperiod control in some long-day plants; for example, eg spinach shows a greater rate of metabolism under long day, and hence production of bioactive GA_1, correlating with developmental transition from rosette form to stem elongation.

(ii) $GA_{20} \rightarrow GA_1$ is blocked in many dwarf (short internode) mutants, such as *le* in pea, *d1* in maize and *dy* in rice. GA_{20} itself is inactive but becomes active after addition of an hydroxyl (–OH) group to the 3β position, thus forming GA_1. In some other dwarf mutants, the pathway is blocked at steps well before GA19, and shoots of these plants contain almost no detectable gibberellins.

(iii) $GA_1 \rightarrow GA_8$ is a key reaction regulating amounts of active gibberellins. In this case, addition of an –OH group to the 2β position inactivates almost every gibberellin, including GA_1.

(d) Structure of abscisic acid (ABA).

amino acid conjugates (e.g. 9-alanyl zeatin = lupinic acid) or from action of degradative enzymes such as cytokinin oxidase which cleave the side-chain.

Gibberellins

Gibberellins were first noticed through symptoms of a disease (known in Japanese as 'bakanae' = foolish seedling) on rice that caused excessive stem elongation. The causative agent, a fungus called *Gibberella fujikuroi*, contains several different types of gibberellin (abbreviated to GA, after gibberellic acid, the first form discovered), some of which also exist in plants. Plants possess many other unique gibberellins, and collective-

ly there are well over 100 identified compounds. This intimidating complexity can be reduced to a comprehensible level by realising that each species contains only about 25 of these gibberellins and that most gibberellins are biosynthetic intermediates or inactive end-products, and not active in their own right. There are many steps and enzymes involved in building up the 19- and 20-carbon gibberellin molecules from five-carbon mevalonic acid. Several parallel pathways exist differing only in number of hydroxyl (–OH) groups. Hydroxyl groups are the key to gibberellin functions: some positions (3β) are generally essential for activity, whereas others (2ß) completely abolish it (Figure 9.1c). Inactivation by conjugation to glucose also occurs. Gibberellin synthesis takes place mainly in developing leaves and stems, in developing seeds and during germination. Gibberellins function in dormancy release and germination, as well as in growth promotion (e.g. stem elongation, fruit tissue expansion).

Abscisic acid

Abscisic acid (ABA) is an unfortunate name because this hormone has little to do with abscission. But once again it tells a story: cotton, one of the plants originally studied, turns out to be an exception in that ABA *does* promote fruit shedding (Okhuma *et al.* 1963). ABA is a 15-carbon molecule (Figure 9.1d) and its synthesis occurs from breakdown of carotenoid pigments, especially violaxanthin, a 40-carbon molecule. Previously, mevalonic acid was thought to be the main precursor, with early steps in common with gibberellin biosynthesis. This alternative pathway may operate in tissues such as avocado mesocarp and in tomato seedlings (Milborrow 1983; Willows *et al.* 1994). ABA is produced in large quantities in water-stressed tissues, especially roots and leaves, but also has a role in seed maturation, dormancy and senescence. ABA concentrations are lowered by oxidative deactivation to phaseic acid or by formation of glucosides.

Ethylene

Ethylene (= ethene; C_2H_4) is a unique gaseous hormone that diffuses rapidly out of plant tissues. Its immediate precursor is 1-aminocyclopropane-1-carboxylate (ACC) which in turn originates from S-adenosyl methionine, a derivative of another common amino acid.

> methionine → S-adenosyl-methionine → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene
> *Enzymes: SAM synthase ACC synthase ACC oxidase*

Ethylene is produced in response to cell damage and other stresses such as anoxia. It accumulates rapidly during fruit ripening and senescence, but all living cells produce some ethylene. Oxidation and conjugation can occur, but dissipation into the atmosphere is probably the main 'means of disposal'.

9.12 How mobile are plant hormones?

In addition to biochemical control of synthesis and inactivation, hormone concentrations can be modified by import and export between different regions of the plant. Indeed, transport is an essential component of long-distance signalling systems. All plant hormones, being small molecules, can diffuse within and between cells. Some may pass readily across lipid membranes; others such as glucosides are more water soluble and may tend to accumulate in the vacuole, along with other cellular waste products. There is probably little a plant can do to prevent local diffusion of hormones, and plasmodesma bridges (Section 10.1.2) allow intercellular cytosolic passage of most hormone-sized molecules. In addition, xylem and phloem sap analysis indicates that several hormones also move over much greater distances, for example perhaps 100 m from deep root tip to leaf of a large eucalypt.

Are mass-flow systems good channels for signal transport? Xylem flux varies massively on a diurnal basis as stomata generally open during the day and close at night, thus modifying transpiration rates. Superimposed on that are seasonal changes in temperature and water availability: with dry roots come slow flow rates; with hot, dry air, there is huge evaporative demand, and rapid sap flow subject to access to a water supply. Likewise, phloem flow is highly variable and sometimes bidirectional, making it difficult to specify source and target. A growing leaf will initially import sugars through the phloem, but with attainment of photosynthetic competence, it will instead export through the same channels. It sounds fraught with potential problems, but most physiologists believe that long-distance transport of hormones has functions in many regulatory processes. Consider that plants have evolved with variable mass flow: perhaps some hormone signalling is *dependent* on such oscillations rather than being defeated by them. There is evidence from tomato plants that xylem ABA

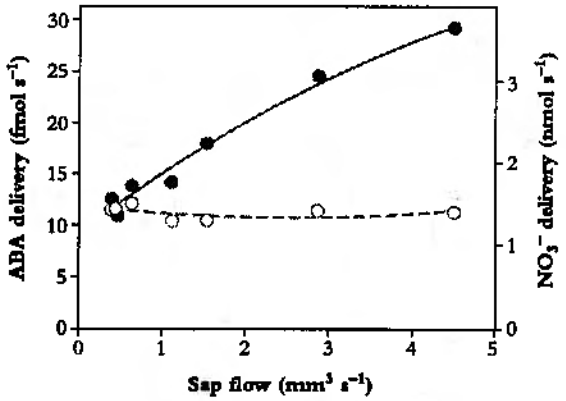


Figure 9.2 Long-distance transport of hormone signals through mass flow can be influenced by sap flow rates. Here, flux in tomato xylem is expressed as a delivery rate (molecules per second) measured as sap flow is altered by pressurising the root system. Flux of ABA in the xylem stream increases with sap flow, whereas flux of nitrate, a major inorganic nutrient, is constant. (Reproduced, with permission, from Else *et al.* 1995)

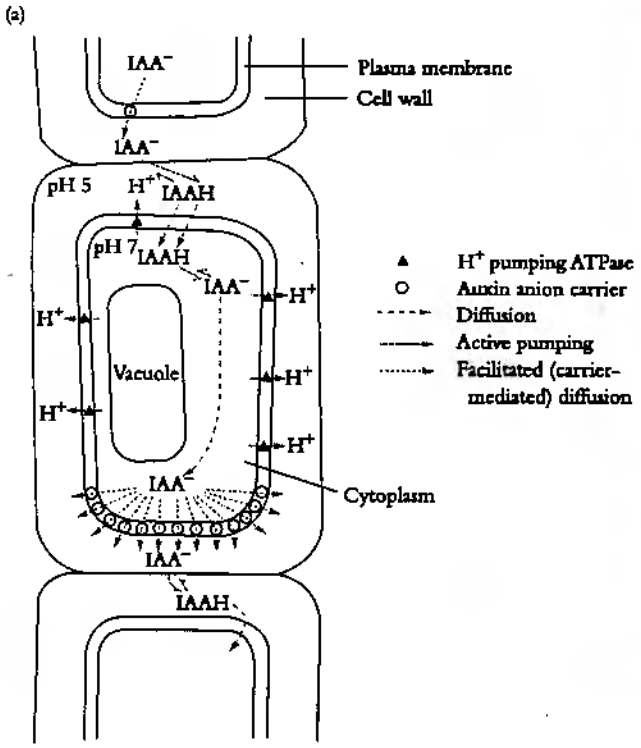
flux (molecules delivered per hour) is influenced by the carrier solvent (sap) flow rate whereas flux of soil solutes such as NO_3^- is independent of flow rate (Figure 9.2; Else *et al.* 1995). In Section 9.2.2 we look further at fluctuations in xylem ABA and the consequences of ABA delivery from root to shoot. Here, we examine specific mechanisms for auxin transport.

Auxin polar transport

The best-studied aspect of hormone mobility is auxin polar transport, the only specific system presently known for movement of any plant hormone. It is termed polar because of its intrinsic directionality which is not altered even by drastic experimental procedures such as excision and tissue inversion. The phenomenon is commonly demonstrated in segments of young shoot tissues such as hypocotyls and cereal coleoptiles in which applied radioactive auxin moves from tip to base at about 1 cm per hour, but hardly at all in the opposite direction. This speed is faster than diffusion but slower than phloem sap flow. Not all cells within the tissue exhibit polar transport, and it is often restricted to ancillary cells within the vascular bundles such as phloem parenchyma. The mechanism of movement is chemiosmotic rather than active, and depends on three factors:

1. the dissociation kinetics of IAA between its neutral IAAH and anionic IAA^- forms;
2. a pH difference between cell wall and cytoplasm;
3. selective IAA! channels in the plasma membrane.

Figure 9.3(a) illustrates how IAA^- ions can pass through the normally ion-impermeant membrane via the IAA^- channels located predominantly in the basal membranes of the transporting cell files. In the cell wall compartment, IAA^- reassociates to IAAH due to the low pH and so the IAA does not readily re-enter the cytoplasm. Activity of the auxin channel protein is blocked by certain synthetic compounds such as naphthyl phthalamic acid (NPA) and tri-iodobenzoic acid (TIBA), as well as by natural plant flavonoids such as quercetin, and apigenin (Jacobs and Rubery 1988). This raises the intriguing possibility that plants may use these natural inhibitors to regulate their own auxin transport. Using antibodies against the protein to which NPA binds, immunofluorescence microscopy has shown that this protein, which is probably the auxin channel itself or a closely associated protein, is predominant in the plasma membrane at the basal end of cells (Figure 9.3b). Because IAA molecules will exit more through these channels, there is a net movement of auxin from top to base of the tissue. The relatively slow speed probably reflects the fact that each IAA molecule has to enter and exit every cell on its route down the tissue.



9.2 Physiology of hormone action

9.2.1 Signal targets: perception and signal transduction

Having outlined how hormonal signals are produced and transported, we turn to further equally important questions: how do cells detect the presence of hormones, and so perceive

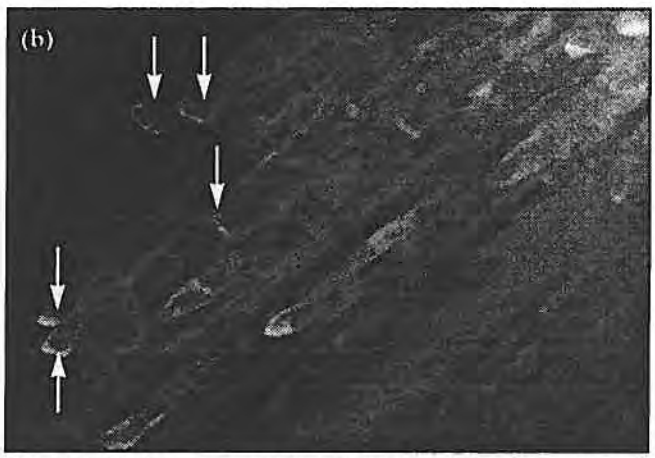


Figure 9.3 Auxin movement in plants operates partly through a polar (unidirectional) transport system. The acidic properties of auxin, together with a pH difference between cell wall and cytoplasm, and localised auxin anion efflux carrier channels in the plasma membrane, combine to generate a net basipetal (away from shoot tip) movement of auxin. (a) Diagram showing the components of this 'chemiosmotic' tranport system. (b) Immunofluorescence micrograph showing presumed location of auxin channels in basal ends of vascular parenchyma cells (bright zones marked by arrows), cut in longitudinal section. The antibody used was raised against purified NPA-binding protein. NPA is an auxin transport inhibitor.
(Reproduced, with permission, from Jacobs 1983 and Jacobs and Gilbert 1983)

changes in hormone concentrations? And then, having measured the signal strength, how is this information converted into developmental and biochemical responses? For the first clue, we turn back to the signals themselves: we know that only certain molecular structures are biologically active and small changes in these molecules can render them virtually inactive. This happens with addition of hydroxyl groups to certain positions of a gibberellin molecule, or by comparing *trans*-zeatin with its *cis* isomer (Figure 9.1). This tells us that the mechanisms for detecting hormones must have great discriminatory powers. Hormone detection involves specific proteins known as *receptors*, proteins being the only class of biological macromolecule that can generate the required precision of molecular shapes. Within their three-dimensional structures, receptor proteins have regions which can bind the active hormone. These *binding sites* are similar to those in enzymes which bind a substrate, the familiar 'lock and key' analogy. The difference with receptors is that we think no chemical reaction occurs during perception; the hormone remains as hormone throughout. This is an important test in receptor assays which usually involve a radioactive hormone: if the hormone is converted to other compounds, probably the binding activity is simply to the active site of a hormone-metabolising enzyme.

(a) Plant hormone receptors

Plant hormone receptor research was neglected for many years but has attracted renewed interest with the advent of new assays and molecular biology tools since the late1980s. Compared with detailed information on control of abundance and activities of mammalian hormone receptors, the picture in plants is sketchy. The best-studied systems have been auxin and ethylene receptors, and some of the genes that code for receptor proteins have been isolated. For example, the *ETR* gene from *Arabidopsis* was suspected to be an ethylene receptor, but this was only confirmed when the cloned gene was expressed in yeast in which the ETR protein was able to bind ethylene (Schaller and Bleecker 1995). There is also new evidence for gibberellin and ABA receptors, but little definitive information on cytokinins. Most of the receptors appear to be located on plant membranes, especially the plasma membrane, and this is also common in animal systems. Some elegant work by Hooley *et al.* (1991) strongly suggests that the gibberellin-binding site is located on the outer face of the membrane, so that it actually picks up hormone signals outside the protoplast. In one experiment, Hooley *et al.* synthesised gibberellin molecules anchored to large Sepharose beads which were incapable of entering the cell, but which still stimulated α-amylase production in protoplasts prepared from germinating cereal seed aleurone cells (Figure 9.4(a). α-amylase is an enzyme responsible for hydrolysing starch to sugars and hence supplying germinating seeds with carbohydrate for energy and growth. In another experiment, Hooley and co-workers generated antibodies that mimicked the shape of gibberellin molecules (known as anti-idiotypic antibodies). These would be recognised by the gibberellin receptor binding site and therefore competed with the gibberellin molecules and interfered with amylase production. The bound antibody molecules were also able to agglutinate protoplasts (Figure 9.4). Taken together, these lines of evidence indicate gibberellin perception occurring at the plasma membrane surface.

Treatment	Response (% of normal maximum)	
	Aleurone protoplasts	Intact aleurone cells
10^{-3}M GA_4-Sepharose	100	11
10^{-4}M GA_4-Sepharose	85	10
10^{-4}M GA_4-Sepharose + 10^{-6}M free GA_4	100	91
Sepharose only	2	9
No additions	1	2

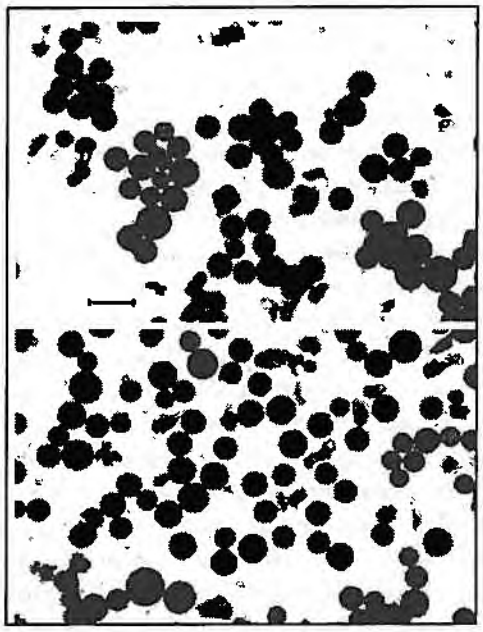


Figure 9.4 Gibberellin receptors in cereal aleurone cells are almost certainly located on the plasma membrane, facing outwards. Two lines of evidence support this view, both making use of protoplasts, that is, cells with their walls enzymatically removed. (a) Gibberellin molecules covalently anchored onto Sepharose (a polysaccharide gel) beads were still effective at inducing α-amylase synthesis. The Sepharose beads were much too large to enter the protoplast. The conclusion is that aleurone cells have receptors that can perceive gibberellin arriving from outside the cell. (b) Antibodies generated that mimic the shape of gibberellin molecules, known as anti-idiotypic antibodies, caused protoplasts to agglutinate. The explanation is that the antibodies are sticking to the outward-facing gibberellin receptor binding site on the plasma membrane. Because each antibody molecule has two binding domains, they can cross-link between cells, so forming aggregates of cells
((a) Reproduced, with permission, from Hooley *et al.* 1991 (b) from Hooley *et al.* 1990)

(b) Selective signal transduction pathways are needed to generate 'right' response

There is now the question of how a single hormone can be involved in several unrelated processes within the same plant. How do tissues stipulate the right response, at the right level and time? There are two main possibilities for ensuring that a signal is passed down the appropriate channel. Note that it is sometimes argued that plants may have only limited control over hormone production and supply and that the signals move and even act in a quite unpredictable manner throughout the plant.

Idea 1

For each response, there is a discrete type of receptor. Bearing in mind the many responses to each hormone, the total number of receptor forms would be high, but this does not necessarily rule out the idea. For example, there are at least five different genes for ACC synthase and three for ACC oxidase, both key enzymes in ethylene biosynthesis (Barry *et al.* 1996; Olson *et al.* 1991; Yip *et al.* 1992). Each member of the *gene family* is regulated by a different set of factors; thus some operate only in ripening fruit, others are induced by O_2 deficit, others are switched on by auxin or by wounding. This division of labour at the hormone biosynthetic level may likewise occur in receptors, as shown by sequence homology between the ethylene-binding protein gene *ETR1* and at least two other genes in *Arabidopsis* and five in tomato (Chao *et al.* 1997; Payton *et al.* 1996). Circumstantial support for multiple receptors also comes from the wide range of effective plant growth regulator concentrations, for example 10^{-10} M auxin promotes root elongation, compared with 10^{-6} M for the same process in shoots, and $>10^{-4}$ M stimulates adventitious root initiation in stem cuttings. How could a single protein have the kinetic power to resolve concentration differences over more than a million-fold range? Auxin inhibition of growth may operate via ethylene because auxin at moderate to high concentrations induces ethylene synthesis (Sakai and Imaseki 1971). However, several other auxin responses are known to be ethylene independent based on studies with auxin-overproducing *Arabidopsis* plants crossed with ethylene-insensitive mutants (Romano *et al.* 1993).

Idea 2

There are very few receptor types, possibly just one, for each hormone. The divergence of signalling would therefore occur 'downstream' from the receptor, that is, events that occur *after* hormone perception. There are several steps between reception and the final action, say, in closing a stomatal pore, inducing onset of dormancy or modifying a cell's growth rate. In this way, a single ABA receptor could be coupled to different responses in guard cell, maturing seed and growing leaf, respectively.

Viewing the collective evidence, a tentative impression would be that perhaps both the above ideas are at least partly correct. Whatever the details of the system, in all cases the signal needs to be converted into a response. After perception by the receptor, the 'signal' is passed to what is commonly termed a *second messenger*, which in animals can be simple molecules such as Ca^{2+} ions, cyclic AMP (cAMP) or inositol trisphosphates (IP_3). These are collectively involved in *signal transduction pathways* and usually involve some kind of amplification: from each hormone molecule binding to a receptor, many second messenger molecules may result. Typically, activation of a single enzyme molecule leads to generation of many product molecules, or opening of a single Ca^{2+} ion channel leads to flux of large numbers of Ca^{2+} ions. Animals and plants share some similarities in signal transduction mechanisms. Membrane-bound mammalian receptors are often linked to other proteins which, for example, bind GTP. These *G-proteins* are linked in turn to enzymes such as phospholipase C, which cleaves phospholipid groups and thus generates lots of second messenger product (IP_3 and diacylglycerol) for as long as the receptor binding site is occupied. The number of G-proteins known in plants is increasing rapidly and they appear to have diverse roles in signalling (Millner and Causier 1996), including hormone systems such as ABA-regulated gene expression in germinating cereals (Bethke *et al.* 1997). In one of the best-studied hormone signalling systems, gibberellin induction of I-amylase gene expression in cereal aleurone cells, there are six or seven stages between primary signal and final action, namely production of active α-amylase enzyme (Figure 9.5; Bethke *et al.* 1997). Within minutes, ionic changes (Ca^{2+} and pH) are detectable in the cytoplasm, followed by an increase in calmodulin, a calcium-binding protein involved in signal transduction. A slower increase in cGMP is seen which seems to operate independently of the Ca^{2+} system, but both of these transduction pathways seem to combine to stimulate transcription of genes via a protein called GAMyb, which is a transcription factor (a protein class that binds to specific DNA sequences in gene promoters). Overall, it takes 4–12 h before much functional α-amylase is detectable.

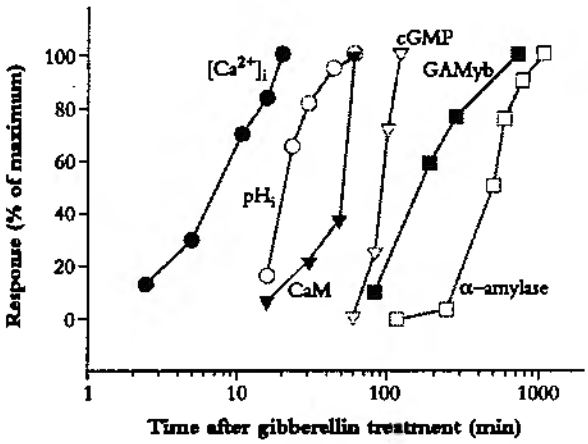


Figure 9.5 A complex chain of events is required to convert a primary hormonal signal, gibberellin, into its final action, in this case synthesis of the starch-hydrolysing enzyme α-amylase during cereal seed germination. After increased gibberellin levels are perceived, the most rapid changes, within 5 min, are in the second messenger Ca^{2+} ($[Ca^{2+}]_i$ is cytosolic calcium concentration) then its receptor protein calmodulin (CaM), together with altered intracellular pH, and after about 1 h another second messenger, cGMP. Following this, at about 3 h, there is a rise in GAMyb, a transcription factor protein that binds to promoters of gibberellin-regulated genes, and finally appearance of I-amylase enzyme about 8 h after gibberellin treatment. (Based on Bethke *et al.* 1997)

A lengthy debate on whether cAMP, a ubiquitous second messenger in animal systems, was important to plants was finally resolved by conclusive data showing presence not only of cAMP but also some of the proteins with which it interacts during auxin-induced cell division (Trewavas 1997; Ichikawa *et al.* 1997). The multiplicity of signalling components in plant

cells and the number of potential links and interactions are beyond the scope of this discussion. What is remarkable is that primary signals are ultimately coupled to the 'right' response, whether direct physiological changes or altered gene expression. Much of this fidelity depends on restricted intracellular distribution of signalling components. For example, many of the protein kinases and protein phosphatases that activate and deactivate other regulatory proteins are probably located on membranes or tied to the cytoskeleton and therefore will only respond to signals within their immediate cellular neighbourhood (Trewavas and Malhó 1997). On top of that, cell differentiation will lead to quite different signalling components in each cell type. It may, however, be some time before enough is known about these subtle systems to be able to make use of them, say, in modifying crop physiology.

9.2.2 Diverse roles for plant hormones

Before we consider details of the final consequences of hormone signalling pathways, it is helpful to think broadly about the roles of hormones in enabling organised plant development and efficient responses to alterations in the environment. The range of functions of plant hormones and responses to them can be bewildering, but most roles can be grouped under two general headings: *organisers* and *mediators*.

(a) Organisers

Organisers primarily define the basic framework of a plant's axial structure. This includes channelling of cells into particular pathways of differentiation depending on their location within the plant. Plants tend to, even need to, maintain a balance between shoot and root development. The two systems are interdependent, so damage or loss of one upsets that balance and necessitates some developmental adjustment. We do not know exactly how complex differentiation pathways are regulated, but it seems likely that a relatively small number of primary signals are needed, with each controlling a whole suite of characters. In Section 9.2.3, we see how such systems can operate at the level of gene expression.

Auxin–cytokinin balance: opposing directional flows of active signal

Tsui Sachs and Kenneth Thimann in 1967 proposed that shoot apical dominance is governed by auxin–cytokinin balance. Evidence came mainly from polar basipetal transport of shoot tip auxin and responses to applied auxins and cytokinins: auxin supplied to a decapitated shoot stump suppresses the normal lateral bud growth response, but cytokinin supplied directly to lateral buds promotes outgrowth of intact plants. More recent studies on transgenic plants and branching mutants suggest that there are probably other regulatory signals in addition to auxin and cytokinin (see Feature essay 9.1). Overall, however, this simple theory, along with auxin and cytokinin responses in tissue cultures (see Section 10.2 and Figure 10.23), gives us the foundations of control of root:shoot development ratios, and enables comprehension of plant developmental homoeostasis. This balancing act comprises several developmental elements, but all potentially trace back to auxin:cytokinin concentration ratios. Even in intact plants, shoot branching is limited if root growth is poor or if roots are stressed. Conversely, a vigorous root system depends on carbon supply from shoot photosynthesis. Superficially, root:shoot balance appears resource limited, but the role of hormones overlays an ability to signal in advance of crisis, and enables stochastic (modular) adjustment of units of plant development: number of active shoot branches and number of lateral roots, in addition to modification of the growth rate of each. Mechanical damage, whether removal of just a shoot apex, or cutting off a stem at ground level, may invoke similar hormone-driven responses. Shoot apex replacement is rapidly achieved by outgrowth of an existing lateral meristem, in theory stimulated by auxin depletion and cytokinin accumulation around the top of a cut stem. A tree stump lacking reserve buds may still possess active cambium cells, which can respond to the same cytokinin enhancement by initiating rapid cell division and subsequent shoot organogenesis (see section 10.1.2). A stem cutting continues to transport auxin to the cut base, but lacks its normal cytokinin supply from the roots. This high auxin:cytokinin ratio stimulates cell activation leading to adventitious root organisation, and in commercial propagation is accelerated by supplying additional auxin in rooting powders and solutions. In addition, there are strong links between cytokinins and delay of senescence (Gan and Amasino 1996; Wang *et al.* 1997). A plant with damaged, diseased, water-stressed or mineral deficient roots will export less cytokinin in the xylem, one consequence being premature leaf senescence usually from the stem base upwards.

Seed-derived hormones regulate pattern of fruit development

Although fruit and seed tissues are genetically different — the former is parental, the latter is progeny — the two develop in a coordinated manner. In most species, if ovules are not fertilised or the embryo aborts, fruit tissues stop growing and are usually shed prematurely. Because seeds represent the next generation, it makes little sense for a plant to continue investing resources in a package that contains no propagules. Exceptions to this are parthenocarpic (seedless) fruits, some of which have genetic causes, and others which are induced chemically by application of growth-promoting hormones: auxin, gibberellin, cytokinin or combinations of these. Parthenocarpic fruit are prized by humans and include seedless or semi-seedless commercial varieties of grape (see frontispiece to Chapter 11), citrus, banana, watermelon, pineapple and lychee. The relationship between seed and fruit growth appears to be driven by hormones synthesised in the developing endosperm and embryo, and is neatly illustrated by the relationship between auxin levels and fruit growth rates in blackcurrant (Figure 9.6).

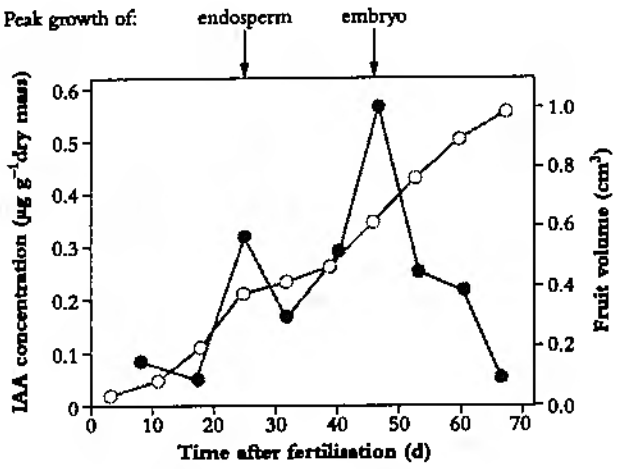


Figure 9.6 During seed and fruit development in many species there is a phase of endosperm development followed by embryo growth. These two tissues are thought to be the source of hormones that promote fruit growth. Here, the two rapid phases during the double sigmoid fruit growth curve of blackcurrant (*Ribes nigra*) coincide with peaks of IAA content (solid circles) and with maximal rates of endosperm then embryo development (open circles).
(Based on Wright 1956)

(b) Mediators

The second broad role of hormones is as mediators of environmental signals, often stress factors, which lead to modification of physiology and patterns of development. For example, as discussed in the preceding chapter, photoperiod perception in leaves leads to flowering at the shoot apex. A light signal is translated into a chemical one. The route of signal transmission from leaf to apex appears to be in the phloem sap, and although we do not know of a universal 'florigen' hormone, part of the signal in some species may be specific types of gibberellin (Evans *et al.* 1994b,c). Gibberellins also have a wider role in mediating some types of phytochrome responses, such as stem elongation in long-day plant rosette species (Wu *et al.* 1996). Entry into and exit from dormancy often depends on external inputs such as fluctuation in water availability: some responses to dehydration during seed maturation and imbibition during germination are mediated by ABA and gibberellins, respectively. Low temperature is another factor which can break endodormancy or induce flowering, and may be mediated by hormones such as gibberellins and cytokinins.

FEATURE ESSAY 9.1 Models for control of shoot branching: more than just auxin and cytokinin

C.G.N. Turnbull

The conventional view of apical dominance control in plants is that auxin inhibits branching whereas cytokinin promotes it. The original paper by Sachs and Thimann (1967) examined responses to auxin applied to cut shoot stumps or cytokinin applied directly to buds. Radiolabelled auxin applied to a shoot stump is transported in a polar manner down the stem (Figure 9.3). These data were extrapolated to the assumption that endogenous hormones will behave similarly, that is, on decapitation (removal of the shoot tip and hence a main source of auxin) auxin supply down the stem will diminish and bud growth is permitted. However, endogenous IAA and cytokinin levels in buds both *increase* within a few hours of shoot decapitation (Gocal *et al.* 1991; Turnbull *et al.* 1997). Since the late 1980s, many transgenic plants have been created with altered auxin or cytokinin content. Superficially, the phenotype of these lines supports the auxin–cytokinin hypothesis: high-cytokinin *ipt* plants branch more, as do low-auxin *iaaL* plants (Medford *et al.* 1989; Romano *et al.* 1991). However, closer examination of the developmental sequences reveals that often branching is hardly affected at all in young plants, even though the constitutively expressed genes cause altered hormone content at all stages of the life cycle. Instead, branching is promoted only later in development, around the time of floral initiation when wild-type plants also branch. Auxin and cytokinin therefore appear to modify rate of bud growth but not always the timing of its onset. In addition, there are questions about whether roots are the main source of cytokinins for the shoot: in experiments with grafted transgenic plants expressing the *ipt* cytokinin gene only in the roots, no increase in shoot cytokinin was seen and plants did not show enhanced branching (Faiss *et al.* 1997).

There is also evidence from pea mutants that branching control in intact plants cannot be explained by auxin and cytokinin alone. The *ramosus* (Latin for 'branched', abbreviated to *rms*) mutants all exhibit greater than normal branching. From Sachs and Thimann, we predict either low auxin or high cytokinin or both, or altered sensitivity to these hormones. However, analysis of xylem sap (the main pipeline supplying solutes including cytokinins from root to shoot) reveals that in some mutants, xylem cytokinin content is actually much reduced, by as much as 40-fold in the case of *rms4*. In addition, none of the mutants has reduced auxin content in the shoot. One mutant, *rms2*, does have slightly elevated xylem sap cytokinin, but it has up to five times the normal auxin level. Clearly, auxin and cytokinin levels do not conform with Sachs–Thimann predictions in these plants, so another model for branching control needs to be developed.

Using reciprocal grafting experiments, Beveridge *et al.* (1997a,b) have established where in the plant some of the *Rms* genes are expressed. For example, expression of the normal *Rms1* gene does not seem to be restricted to the shoot. This conclusion is based on inhibition of branching

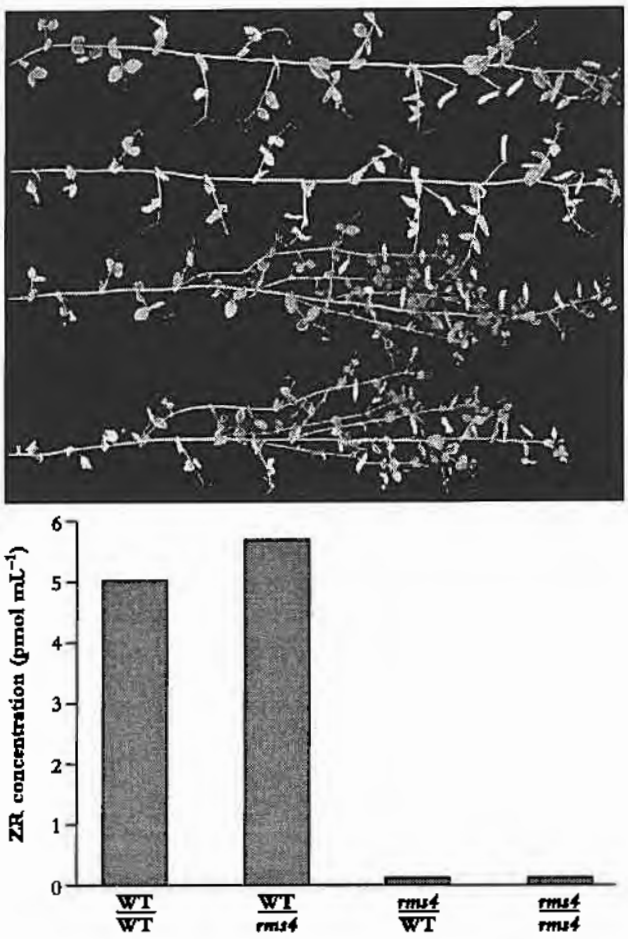


Figure 1 Control of lateral branching is regulated by several genes and probably several mobile signals. In pea, the *rms4* mutant is highly branched and has extremely low levels of cytokinins moving from root to shoot in the xylem sap. The conventional theory of apical dominance regulation suggests that *high* cytokinin levels would be associated with increased branching. The evidence here from reciprocal grafts between *rms4* and its wild type is that the extent of shoot branching governs the export of cytokinins from the root rather than vice versa. ZR = zeatin riboside

(Reproduced, with permission, from Beveridge *et al.* 1997a)

in both *rms1*/wild-type (scion on rootstock) and wild-type/*rms1* grafts; that is, provided there is one part of the plant with normal *Rms1* expression, then branching will be inhibited compared with the *rms1* mutant. The *Rms1* gene therefore may control a branching inhibitor that can move from root to shoot, but can also act directly in the shoot. Because *rms1* plants have normal IAA transport and are not IAA deficient, we deduce that this inhibitory signal is almost certainly not auxin.

On the other hand, *rms4* shoots grafted onto wild-type roots still branch, but the wild-type roots now export *rms4* levels (i.e. very low) of cytokinin. The converse graft of wild-type shoots onto *rms4* mutant roots does not branch but the roots now export wild-type levels of cytokinin (Figure 1). The deduction is that the normal *Rms4* gene is expressed in the shoot only, and two consequences of the *rms4* mutation are enhanced branching and down-regulation of root cytokinin export. The latter must require a shoot to root signal, but auxin is again an unlikely candidate. We are therefore left with two intriguing conclusions:

1. Auxin and cytokinin alone do not explain the control of apical dominance in intact plants.
2. there is evidence for at least two novel (i.e. not auxin or cytokinin) graft-transmissible branching signals, one moving from root to shoot (the *Rms1* factor) and one moving from shoot to root (a signal relating to *Rms4*).

Plant architecture is closely tied to shoot branching and is an important character in many crop plants. For example, increased bushiness is desirable in ornamental pot plants, but a single trunk, non-branching phenotype is most valuable in plantation timber species. In the future, there may be potential for regulating branching through genes such as the *Rms* series, in addition to manipulation of auxin and cytokinin status.

References

Beveridge, C.A., Murfet, I.C., Kerhoas, L., Sotta, B., Miginiac, E. and Rameau, C. (1997a). 'The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant rms4', *Plant Journal*, **11**, 339–345.

Beveridge, C.A., Symons, G.M., Murget, I.C., Ross, J.J. and Rameau, C. (1997b). 'The rms1 mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased branching controlled by graff-transmissible signals', *Plant Physiology*, **115**, 1251–1258.

Faiss, M., Zalubilá, Strnad, M. and Schmülling, T. (1997). 'Conditional transgenic expression of the ipt gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants', *Plant Journal*, **12**, 401–415.

Goccal, G.F., Pharis, R.P., Yeung, E.C. and Pearce, D. (1991). 'Changes after decapitation in concentrations of indole-3-acetic acid and abscisic acid in the larger axillary bud of *Phaseolus vulgaris* L. Cv Tender Green', *Plant Physiology*, **95**, 344–350.

Medford, J.I., Horgan, R., El-Sawi, Z. and Klee, H. (1989). 'Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene', *Plant Cell*, **1**, 403–413.

Romano, C.P., Hein, M.B. and Klee, H.J. (1991). 'Inactivation of auxin in tobacco transformed with the indoleacetic acid–lysine synthetase gene of *Pseudomonas savasanoi*', *Genes and Development*, **5**, 438–446.

Sachs, T. and Thimann, K.V. (1967). 'The role of auxins and cytokinins in the release of buds from dominance', *American Journal of Botany*, **54**, 136–144.

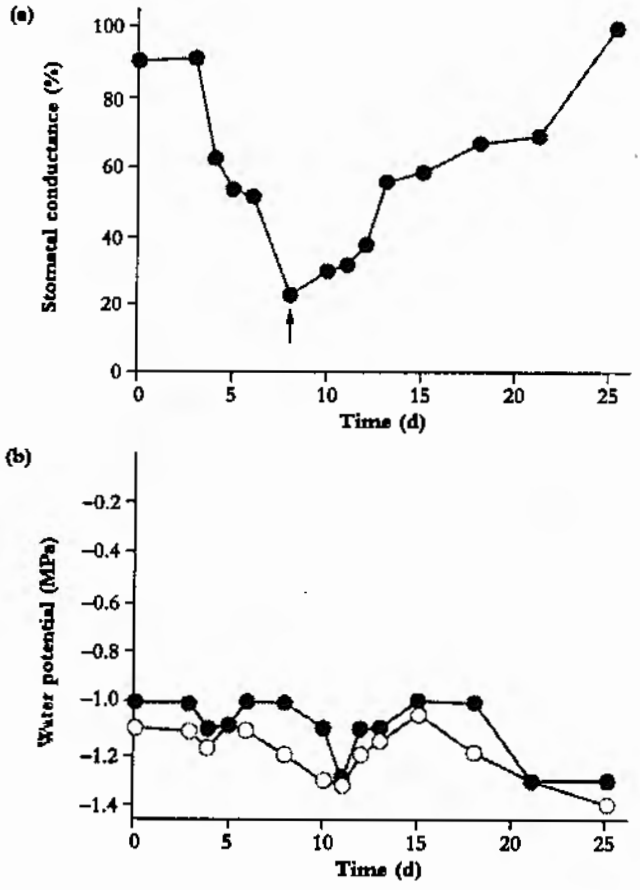
Turrnbull, C.G.N., Raymond, M.A.A., Dodd, I.C. and Mitchell, S.E. (1997). 'Rapid increases in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance', *Planta*, **202**, 271–276.

ABA as a mediator of water status information

The role of ABA in transmitting information about plant water status was discovered in the early 1970s. Here, we take a detailed look at one of the best studied of all 'mediator' roles of hormones. When water loss from leaves is accelerated by exposing them to a stream of warm air, ABA concentration rises dramatically, by about 10- to 50-fold and stomata close (Zeevaart 1980). Similarly, a low concentration of ABA supplied exogenously to excised leaves via the transpiration stream induces stomatal closure. It was concluded that ABA synthesis in leaves, induced by water stress, is the cause of stomatal closure. Further evidence for the involvement of ABA in stomatal regulation came from studies of ABA-deficient mutants such as *flacca* in tomato, *wilty* in pea and *droopy* in potato, which wilt rapidly when exposed to only mildly stressful conditions but regain a normal phenotype if treated with ABA.

Synthesis of ABA in wilting leaves is enhanced as turgor approaches zero. Conjugated forms of ABA such as the J-glucoside can be present in leaves at quite high concentration and represent a potential source of free ABA, but actually appear quite stable and do not break down under stress. Therefore *de novo* synthesis of ABA during stressful conditions is responsible for stomatal closure, and acts as a protective mechanism against the potentially damaging effects of water loss. ABA-induced stomatal closure pre-empts hydraulically driven stomatal closure which would eventually occur as stomatal guard cells lose turgor. Hydraulically driven closure occurs far later than closure induced by ABA and usually occurs too late to prevent excessive and damaging levels of water deficit.

Stomatal closure in response to increased levels of foliar ABA provides a solution to one of the major problems faced by mesophytic plants, that is, the compromise between maintaining sufficient gas exchange to satisfy the CO_2 requirements for carbon assimilation but at the same time limiting water loss when conditions become unfavourable. However, this rather simple interpretation of plant response to stress is not the whole story. For example, water-stressed plants can have leaf water potentials which are similar to or even higher than those of well-watered plants and yet the stomata are fully closed (Figure 9.7). Shoot extension and leaf expansion are



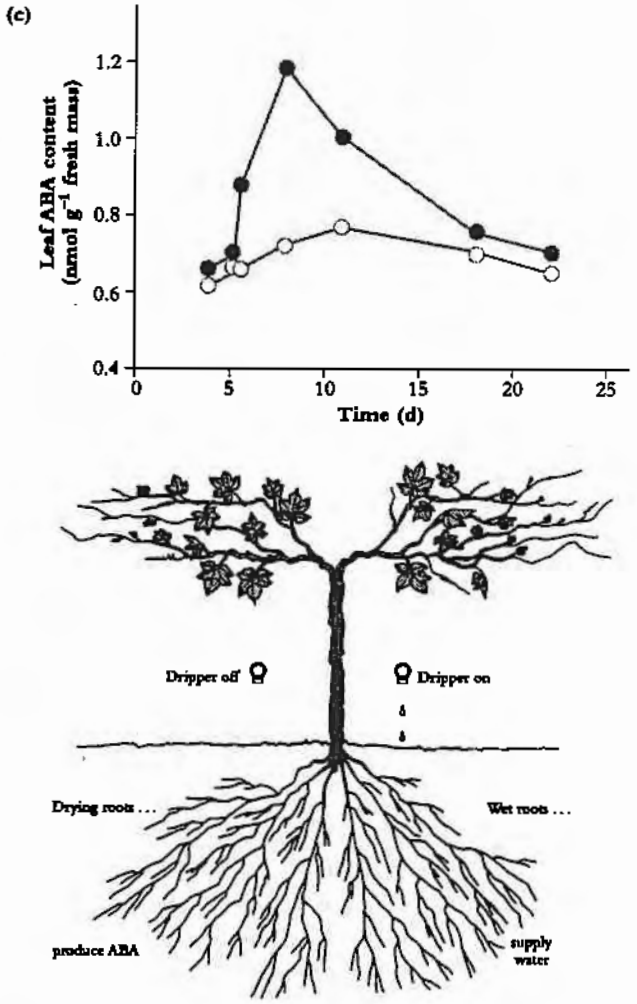


Figure 9.7 Response of grapevines to hydraulic and non-hydraulic signals during water stress applied to split root systems. Roots systems can be split vertically (surface vs. deep) or horizontally (left–right, as in this experiment (d)) and different water stresses applied to each half. If one half is kept well watered, this is sufficient to maintain normal shoot water status and no leaf water potential difference is detected (solid circles) compared with control fully watered plants (open circles) (b). If the other half root system is dry, any drought signal induced may be transmitted to the shoot. A prime candidate for this root signal is ABA, which is detected either chemically (c), or by its effects on stomatal aperture (a). In many species, root-generated ABA can cause stomatal closure in the absence of any water deficit in the shoot. This is interpreted as an early warning system, enabling plants to reduce water use under imminent drought conditions. In nature, during periods without rain, surface roots would normally become dry before deep roots (Diagram courtesy B.R. Loveys)

also highly sensitive to stressful conditions but they are not always accompanied by low leaf water potentials. Clearly, ABA synthesis in leaves is not the only process occurring during water stress.

Roots as a source of ABA

Part of the answer to this puzzle came from experiments using plants with divided root systems. For example, if a piece of grapevine cane bearing six or seven nodes with dormant buds is sawn along its length from the base for about two internodes, it is possible to induce root formation on each of the two halves. These split root systems can be planted in separate pots or in the field, which allows independent manipulation of the soil moisture status of each root (Figure 9.7(d)). It has long been known that xylem sap contains ABA, and that increased ABA in droughted roots might thus be transmitted to the leaves (Davies and Zhang 1991). However, simply drying the roots of a plant with a single root system affects the water relations of the whole plant and it is then difficult to distinguish the effects of lowered leaf water potentials from the effects of root-derived chemical signals. Split-root plants allow study of the effects of drying soil without the complications of changed water relations because the soil around one root system is maintained fully watered. This root system supplies as much water as the canopy needs. Normally, the second root system is then dried and the effects of any chemical signals studied. Here we show the effect of withholding water from one root system of twin root grapevines (Figureure 9.7). Stomatal conductance fell rapidly, and within 8 d was only 22% of that in fully watered control vines yet water potential of the leaves remained unchanged. Leaf ABA content also changed in response to partial root drying. When conductance was at its lowest, ABA levels had doubled when compared with fully watered controls. ABA levels returned to control values 10 d after rewatering the dry pot, but conductance took somewhat longer to recover.

The leaves did not receive any hydraulic (water deficit) signal which may have initiated local ABA synthesis. We conclude that the drying roots produced a chemical signal which is transported to the leaves in the transpiration stream and which induces stomatal closure. The chemical signal is most likely to be ABA. Zhang and Davies (1990) took a direct approach by supplying different solutions to excised maize leaves and measuring stomatal conductance (Figure 9.8). They tested xylem sap from well-watered leaves, sap from stressed leaves, sap from which most of the ABA had been removed by passage through a column containing anti-ABA antibodies and a series of synthetic ABA solutions. In every case, the antitranspirant activity of each solution was explicable in terms of its ABA content. Further evidence that it is a closing stimulus arising from drying roots and not a lack of an opening stimulus comes from the observation that stomata re-open after drying roots are excised.

Experiments like this help us understand how plants in the field deal with everyday fluctuations in soil water, sustained

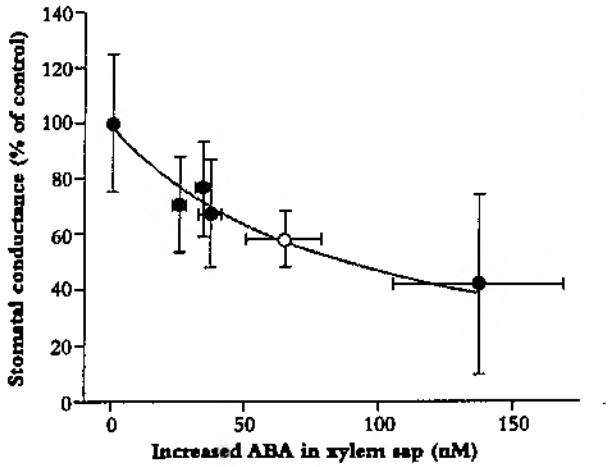


Figure 9.8 Large fluctuations are often seen in ABA concentrations in xylem sap moving from root to shoot. In some species such as maize (but not others such as wheat and apricot; see Figure 9.9), drought-induced stomatal closure can be accounted for entirely by the increased amount of ABA signal. In this experiment, increased ABA was generated by withholding water for up to 20 d. The error bars on each point are standard deviations and indicate the range of both the increase in ABA content and the effect on stomatal conductance. The open circle shows the xylem ABA concentration and leaf conductance resulting from feeding 10^{-5} M ABA to part of the root system.
(Redrawn, with permission, from Zhang and Davies 1990)

drought and other environmental conditions. Surface layers of soil, which usually have the highest root densities, dry first and roots in this zone will be stimulated to send enhanced ABA signals to the leaves, slowing transpiration and thus providing the first indication that soil conditions are not ideal. At this time, the deeper roots may still have access to water and so no hydraulic signal has been generated. Such ABA signalling from roots does not result in large increases in leaf ABA and that which does accumulate is soon dissipated through metabolism and translocation once the dry root signal is removed. This enables leaves to monitor continuously root water stress and to adjust stomatal apertures according to distant and local water availability. If drought continues and more of the soil profile dries, then leaf water potentials will fall and trigger new synthesis of ABA in leaves, reinforcing and extending the stomatal closure already set in train by the roots. The large increases in ABA which then result may have more far-reaching consequences because expression of certain genes is regulated by ABA (see Section 10.3). Some of these have sequences which are predicted to confer heat stability to their resulting protein products.

Unexpected relationships between conductance and ABA content

The picture of ABA derived from roots causing reduced stomatal conductance during periods of water deficit is true in many, but not all, cases. For example, this correlation does not hold in apricot which instead can osmoregulate and thereby maintain stomatal opening. In a study comparing the drought responses of grapevine, which largely conforms to the normal model, with apricot, another drought-tolerant plant often grown in close proximity to grapevine, xylem

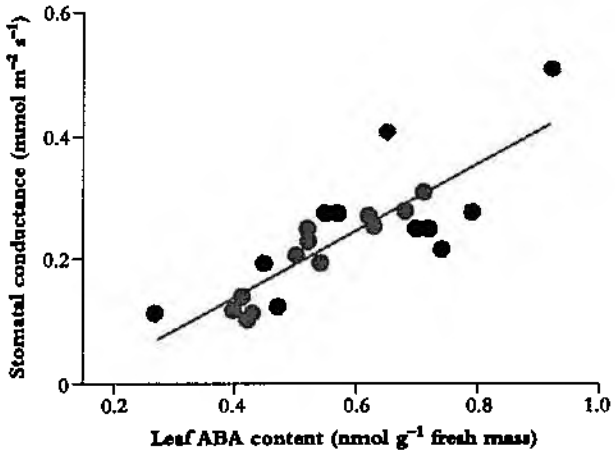


Figure 9.9 In an unusual response, stomatal conductance was positively correlated with leaf ABA concentration and not inversely correlated as expected. This study used apricot plants, which are able to osmoregulate during drought and thereby maintain cell turgor and hence open stomata. The increase in ABA is probably the result of increased supply from the roots during drought, but in this case the ABA does not result in stomatal closure (Reproduced, with permission, from Loveys *et al.* 1987)

ABA content in apricot was only about 5% of that needed to induce stomatal closure. Moreover, ABA levels in leaves showed a positive relationship with stomatal conductance, contrary to normal expectations (Figure 9.9). ABA was accumulating with increased transpiration but was having no effect on stomatal conductance. Unlike grapevine, which operates over a fairly narrow range of leaf water potentials, apricot leaf water potential fell progressively throughout the season, yet stomata remained open. The key to these apparently anomalous results was that leaf osmotic potential fell along with the water potential, thus maintaining leaf turgor and stomatal opening. By the end of the growing season, sorbitol, the major organic osmoticum in apricot leaves, had accumulated to a concentration of 400 mM. Apricot uses osmotic adjustment to protect itself during drought and ABA appears to play little part. Indeed, it was found that even when apricot leaves were deliberately wilted, their ability to synthesise ABA was almost totally eliminated when sorbitol concentrations were high (Loveys *et al.* 1987).

Next, we follow the signalling pathways to their final sites of action, namely modified development and physiology. Essentially, there are two options: direct effects and action through altered gene expression.

9.2.3 Direct effects on cellular processes

Some hormone systems are coupled to existing components of a cell's physiology. This is an effective means of achieving rapid responses, often within a few minutes of alteration of hormone levels. Three cases are presented which illustrate how important direct effects can be.

(a) Auxin and acid growth — the proton pump story

In the 1930s, the original proposed function of auxin was as a shoot growth promoter, also involved in unequal rates of cell elongation in cereal coleoptiles during bending associated with tropisms. We now know that auxin is active in promoting cell expansion in many other tissues: stems, roots, fruits and callus cultures. Two mechanisms seem to be involved, one involving rapid changes in gene expression (see Section 9.2.4b) and another which directly affects the cell wall. This latter may operate through what is often termed the *Acid Growth Theory* and relates to auxin stimulation of 'proton

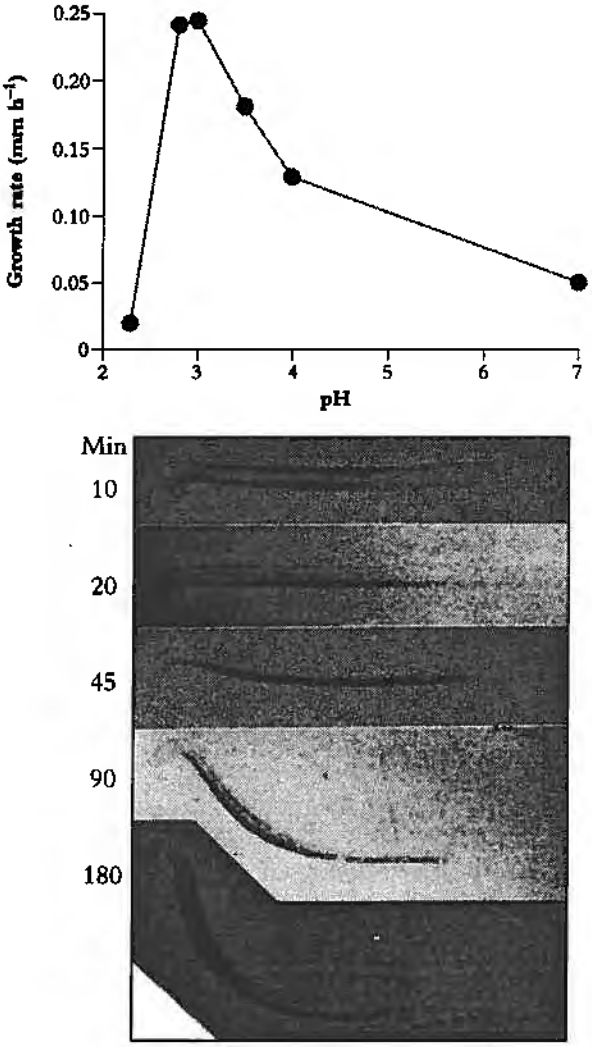


Fig 9.10 Auxin-induced cell elongation operates through more than one mechanism. (a) Short-term growth may partly be due to acidification of the cell wall compartment due to auxin stimulation of plasma membrane H^+ export (proton pump) ATPase. Here, growth rates of oat coleoptile segments were measured during a 45 min incubation in solutions of different pH. (b) Specific auxin-inducible genes are expressed more abundantly in faster growing tissues, with changes in expression detectable within 10 min. These may well be related to auxin-induced growth that occurs independently of, or additively with, acid-induced growth. Here tissue print autoradiograms show distribution of *SAUR* (short auxin upregulated) mRNAs during gravitropic response of soybean hypocotyls. At time 0, the seedlings were moved from a vertical to horizontal position. Initially symmetrical staining is replaced by predominance of staining on the lower (faster growing) side during the bending reponse. The unexpanded cotyledons are just visible at the left-hand side.
Reproduced, with permisson, from Rayle and Cleland 1970 and Guilfoyle *et al.* 1990)

pump' ATPases located in the plasma membrane which rapidly increase H^+ concentrations in the cell wall compartment (Figure 9.10(a); Rayle and Cleland 1970). Low pH was originally proposed to modify some of the bonding between cell wall polymers, especially H-bonds and ionic bonds, and also to stimulate some hydrolytic enzymes. The mechanism now appears to involve cell wall proteins called expansins which are pH sensitive and interact with other cell wall polymers to modify wall mechanical properties (Cosgrove 1997). The result is a softening, or increase in *plasticity*, of the wall which then expands more rapidly under the driving force of turgor. The other component of wall mechanical properties is referred to as *elasticity* but because this is reversible deformation it does not lead to growth. Some controversy has existed on this subject since the 1970s, mainly centred on whether acid growth fully accounts for the auxin effect, and whether it is a universal phenomenon. There is little doubt that auxin stimulates proton pumping and acid-induced growth is probably at least a part of the *initial* growth response, but there are also acid-independent components of growth (Schopfer 1989) and other changes are needed for sustained auxin-induced growth. The discovery of auxin-stimulated genes that respond within 5 min (Section 9.2.4) suggests that direct acid-induced growth and gene expression changes may occur simultaneously.

(b) Gibberellins and ethylene modify growth directions via control of microtubule orientation

Plant shape or form is determined by the directions in which its component organs and tissues grow. Disorganised growth in all three dimensions leads to a callus or tumour, so an organised plant clearly has spatial control of growth. Theoretically, each cell can grow in any direction, but usually the existing cell walls place some mechanical restriction on this. Cellulose microfibrils — bundles of cellulose molecules — contribute a large proportion of the strength of the primary wall, and have only limited elasticity. This means that growth along the axis of the microfibrils is restricted, but growth perpendicular to this axis can continue. Organising microfibrils into parallel arrays will therefore force predominantly one-dimensional elongation growth. Microfibril orientation, in turn, is dictated by subcellular components just inside the plasma membrane called microtubules (see Section 10.1.2). Any factor that modifies the microtubule arrays can alter growth rate or direction. Indeed, gibberellin accelerates elongation rates in stems, associated with more longitudinal microtubules (Figure 10.15), and ethylene leads to radial (swelling) growth because it causes microtubules to take up more random orientations (Figure 9.11). An intriguing exception to this is the rapid internode elongation induced by flooding of semi-aquatic species such as deepwater rice. This adaptation maintains the leaves above the hypoxic conditions within the paddy waters. Indeed, low oxygen concentration is the stress signal that initiates the growth response by inducing ethylene synthesis. What is unexpected is that ethylene in this case does not lead to radial cell expansion but instead to elongation. The explanation comes from evidence that in this species ethylene increases tissue sensitivity to gibberellins, which in turn stimulate greater than normal shoot extension (Raskin and Kende 1984). This is a neat example of interactions between hormones resulting in a much improved adaptation to a specific environmental problem.

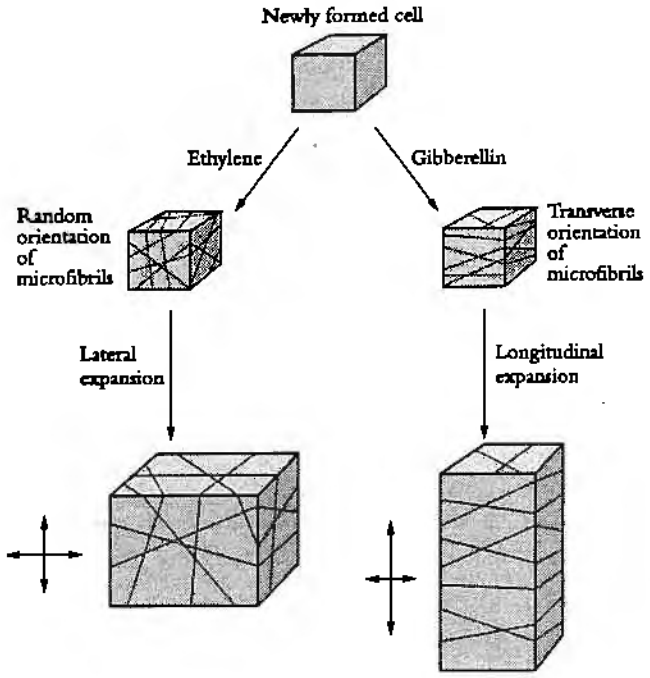


Fig 9.11 Direction of cell growth is influenced by at least two hormones, gibberellin and ethylene, through effects on cellulose microfibril orientation. The microfibrils form the bulk of the strength of primary cell walls. Gibberellins lead to largely transverse orientation, which constrains growth mainly to the longitudinal direction, that is, elongation, whereas ethylene promotes a more random orientation and hence growth in all three dimensions, that is, tissue swelling. In both cases, the positioning of new microfibrils in the cell wall is governed by orientation of microtubules in the cytoplasm just beneath the plasma membrane. The mechanism is described in more detail in Section 10.1.2
(Reproduced, with permission, from Raven *et al.* (1992)

(c) ABA and stomatal guard cells

We previously mentioned the role of ABA in regulating stomatal aperture. This response does not involve growth, it is rapid and reversible, and the magnitude of opening or closing is dependent on ABA concentration across a wide range. Leaf epidermis can be peeled off in a single cell layer and floated on ABA solutions, resulting in a stomatal closure response which commences within minutes. The mechanism does not seem to involve changes in gene expression, at least not initially. Instead, water and solutes are moved rapidly out of the guard cells and the change in aperture is a function of cell turgor and volume. During closure, potassium channels in the guard cell plasma membrane open, allowing potassium ions to flood out into the adjoining subsidiary cells. Anions such as malate and chloride move in the same direction to maintain electrical neutrality. This change in total solute content leads to osmotic imbalance and hence rapid water efflux from the guard cells (Figure 9.12). The resultant cell volume change is

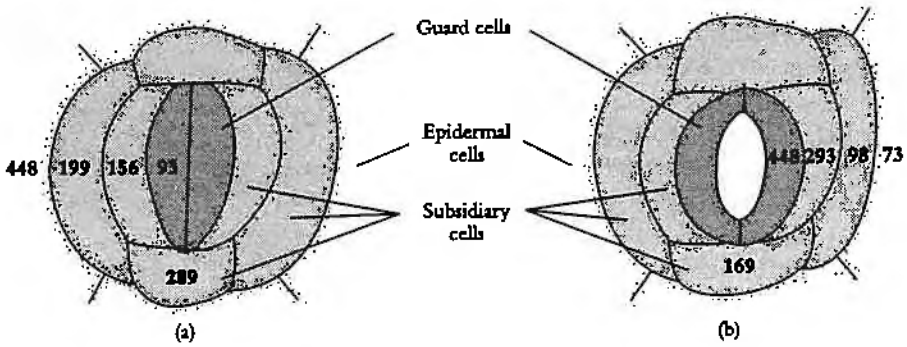


Figure 9.12 Stomatal aperture changes are effected by movements of solutes and water in and out of guard cells, processes influenced by ABA. (a) Without ABA; (b) within minutes of additional ABA being supplied to guard cells, signal transduction pathways operating through Ca^{2+} and other amplification systems lead to massive potassium ion efflux through K^+-specific ion channels in the plasma membrane. Potassium levels in the adjoining cells increase correspondingly (K^+ values are in mM). Anions such as malate and chloride also move, and the net change in guard cell water potential leads to water also moving out of the guard cells by osmosis. The reduction in cell contents lowers the turgor and cell volume, and the stomatal pore closes (Reproduced, with permission, from Raven *et al.* 1992)

the direct cause of stomatal closure. Re-opening tends to be slower, because it takes time for the ABA level to decline and for the solutes to be moved back.

9.2.4 Modified gene expression

With the advent of more sophisticated methods of detecting changes in gene expression, it has become clear that many responses to plant hormones operate through up- and down-regulation of specific genes. In some cases, hormone signals elicit gene expression within a few minutes, so speed of response is no longer a diagnostic tool for deciding whether a hormone is acting directly on physiological processes. Analysis of DNA sequences in the promoters of hormone-regulated genes has shown common short sequences, typically four to twelve nucleotides long, called 'response elements', which are unique to each hormone class and are essential for hormone action (Table 9.2). Provided other essential factors are present, these response elements allow a single hormone potentially to regulate expression of whole suites of genes each carrying the same response element.

(a) Gibberellin- and ABA-induced gene expression in germinating cereals

The 'opposing forces' of gibberellin and ABA in seed dormancy were described in Chapter 8, but these hormones also appear to operate in regulating metabolism in the seed during the germination phase. This is best known in cereal seeds. Gibberellins, produced in the embryo and scutellum, move through the endosperm to the *aleurone layer*, where they stimulate expression of a suite of genes coding mostly for enzymes that hydrolyse stored starch, protein and lipids. These resources are contained in the endosperm into which the enzymes are secreted. The released products move to the embryo and provide it with energy and the building blocks required for the rapid growth of the developing seedling. The best-known genes are those for α-amylase which degrades starch to sugars. If, however, ABA is applied before or with the gibberellin, then the α-amylase gene expression is much reduced. We now know from DNA sequence analysis of the gene promoter in rice, wheat and barley that there are specific response elements upstream from the transcription start (see Section 10.3) which are necessary for gibberellin or ABA to be effective (Table 9.2). If either the gibberellin-response or ABA-response element is excised or deleted from the gene, then the

Table 9.2 Some known promoter elements associated with regulation by plant hormones.

Hormone	Promoter element sequence	Gene	Trancription factor	Source	Reference
Auxin	CCTCGTGTCTC[a]	*GH3*	ARF1	Soybean	Ulmasov *et al.* (1997)
	TGTCTC	*SAUR*		Soybean	Li *et al.* (1994)
Gibberellin	TAACAAACTCCGG	Amylase		Rice aleurone	Tanida *et al.* (1994)
	TAACAGAGTCTGG	Amylase	GAMyb	Barley aleurone	Gubler *et al.* (1995)
	TAACAUANTCYGG	Amylase		Aleurone	Bethke *et al.* (1997)
	YCTTTT	Amylase		Aleurone	Bethke *et al.* (1997)
	TATCCAY	Amylase		Aleurone	Bethke *et al.* (1997)
Abscisic acid	GTACGTGGCGC	*HVA1*		Barley aleurone	Shen and Ho (1995)
	NCACGTGGC	*EM*	EMBP-1	Wheat embryo	Guiltinan *et al.* (1990)
Ethylene	TAAGAGCCGCC	*PRP*	EREBP	Tobacco leaf	Ohme-Takagi and Shinshi (1995)

[a]Underlined DNA sequences are essential for response to the hormone. This is normally deduced from deletion analysis experiments (see Section 10.3) which involve progressive excision of increasing lengths of the promoter until the hormone response is lost. Base codes are adenine (A), cytosine (C), guanine (G), thymine (T), any purine (U), any pyrimidine (Y) and any nucleotide (N)

response to that hormone is lost. The time scale involved in switching on gene expression is around 1 h and measurable increase in enzyme activity occurs soon afterwards (Higgins *et al.* 1976).

(b) Auxin-induced growth genes

Some of the most rapid changes in gene expression yet found in plants are those that occur in response to auxin application to growing tissues. The work of Guilfoyle on the *SAUR* (short auxin upregulated) and *GH3* genes of soybean has shown enhanced mRNA levels within 2–5 min (Guilfoyle *et al.* 1992). We do not yet know exactly what the functions of the gene products are but they are more abundant in faster growing cells, for example on the lower side of a horizontally placed hypocotyl as it responds to light or gravity (Figure 9.10(b)). In this case, no additional auxin was supplied, and the distribution of gene expression may reflect localisation of endogenous auxin. Two interpretation are possible: (1) this is proof that modified endogenous auxin levels are involved in tropisms; (2) this simply demonstrates that auxin-induced and tropism-induced growth stimulate expression of the same growth-associated genes.

9.3 Harnessing hormones: making use of chemical signals

9.3.1 Manipulating growth and development with applied plant growth regulators

Plant growth regulators (PGRs) are a diverse group of chemicals classified by their ability to modify plant development and/or biochemical processes. They include not only the native plant hormones already discussed and their synthetic analogues, but also many other compounds that influence hormone physiology, especially inhibitors of hormone biosynthesis and compounds that block hormone action, perhaps by interfering with receptor binding.

(a) How specific can we be?

From the preceding sections, it is clear that hormones are multifunctional, and responses depend on dose, site of application and developmental stage. In theory at least, we have the potential to influence almost any developmental process, and over the past 60 years probably just about every PGR on the shelf has been tried out. Controlling plant height, inducing flowering, increasing fruit numbers, generating seedless fruit, inducing seed germination — all worthy aims often with commercial success as reward for the 'successful'.

But all is not so simple. For every 100 attempts, probably only one becomes regular practice in agriculture or biotechnology. Why? The multifunctionality, the variability of response between genotypes, between tissues of different age, modification of response by environmental factors — all these can thwart the best-planned strategy even with the 'right' dose, timing and placement on the plant. In pharmacological terms, the side effects are often stronger and more undesirable than the targeted response. Here we select a few examples which have found commercial application.

Stem elongation and gibberellins

Gibberellins are well known for effects on dormancy, germination, flowering and fruit development, but one of their most studied roles is in modifying stem elongation. This is a 'rate' process rather than an 'all-or-nothing' on/off switch, so we predict graded changes in cell growth rates as gibberellin concentrations are modified upwards or downwards. The tools are gibberellin mutants (deficient either in the capacity to produce active gibberellins or the capacity to respond to them; see Section 9.3.2), several gibberellins available in commercial quantities, and several compounds that more or less specifically inhibit gibberellin biosynthesis (Figure 9.13). Thus we have

Figure 9.13 The effect of paclobutrazol, an inhibitor of gibberellin biosynthesis, on shoot growth and flowering of poinsettia, a popular ornamental pot plant. The treated plant on the right has shorter internodes, darker green leaves and slightly enhanced flowering, all characteristics of gibberellin depletion

possibilities of examining the effects of genetically or chemically altering gibberellin levels. The almost universal result is that plants with low gibberellin concentrations end up shorter (more dwarfed) than those with higher levels. There are limits to the range over which this applies, that is, there is a ceiling growth rate beyond which no response is elicited by extra gibberellin, but in the best-studied examples, such as pea, the classic log dose–linear response relationship seems to hold quite well (Figure 9.14).

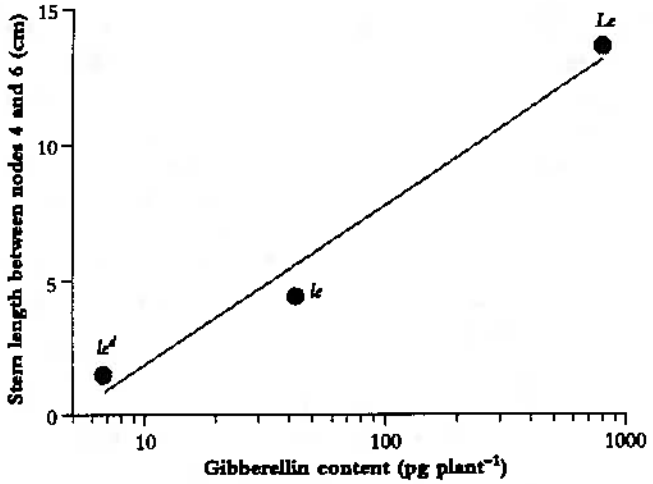


Figure 9.14 Genetic and hormonal control of development are illustrated by the relationship between active gibberellin content and internode length in pea genotypes with different alleles at the *Le* locus, a gene which encodes an enzyme for synthesis of bioactive GA_1. The wild-type *Le* has a normal enzyme and tall stature, *le* is dwarfed and has a defective but leaky enzyme, and *le^d* is extremely dwarfed with almost no enzyme activity. Note the linear relationship between internode length and log of gibberellin content, showing a very wide range of concentrations over which the plant can detect gibberellins. This graph is similar to the classical 'dose–response' plots used in early hormone research to test biological activity of exogenously applied compounds

(Redrawn, with permission, from Ross *et al.* 1989)

Parthenocarpic fruit: auxin and gibberellins

One highly desirable characteristic in most fruit crops (though not in nut crops!) is seedlessness. This occurs spontaneously in banana because of its triploid genotype, and in certain kinds of citrus because of early embryo abortion. In some mandarin types, an auxin spray before or just after bloom induces fruit to set without seeds. In certain grape varieties such as Sultana (known elsewhere as Thompson Seedless) the seed starts to develop but then aborts and added gibberellin is required to promote normal fruit development (see frontispiece to Chapter 11). In both cases, the applied hormone is thought to be substituting for what would normally be generated by the growing seed (Figure 9.6). This gives an insight into how fruit and seed development are intimately coordinated. Auxins can cause similar seedless fruit in other crops such as citrus, but later applications can lead to abscission, probably via induction of ethylene synthesis, and are useful for fruit thinning on trees that otherwise tend to bear excessive numbers of undersized fruits.

Tissue culture: auxin and cytokinins — the essential hormones

When a plant breeder or horticulturalist finds a new, potentially valuable plant, the first priority is usually to multiply it. Often the plant may be infertile or progeny may be genetically inferior. It then becomes necessary to use vegetative propagation. Remember that many plant cells have a remarkable property called *totipotency* (section 10.2), so almost any fragment of a plant (or *explant*) can be used to regenerate new genetically identical plants, called clones. The most dramatic advances in plant propagation, resulting in the ability to generate millions from one, are due to *tissue culture* or *micropropagation*. Not all plants spontaneously enter into useful forms of regeneration when cultured: indeed most need some form of chemical persuasion. The most powerful control comes from use of two hormones, auxin and cytokinin. Auxin tends to promote cell expansion and, together with cytokinins, induces cell division, whereas cytokinins can induce cell division. On top of these fundamentals of tissue growth, auxin causes cells to become organised, sometimes simply into vascular tissue but more importantly to form roots. Cytokinins on the other hand induce shoot formation. The *ratio of concentrations* of auxin to cytokinin, as discussed in Section 9.2.2, appears to determine the type of development that ensues.

Legislation, safety and moral issues

In addition to the physiological issues, increasing awareness of environmental pollution and potential dangers of exposure to hazardous chemicals have led to critical examination of use of all types of chemicals, especially on food crops. Initial concerns were over pesticides, especially organochlorines, but then spread to include PGRs. A few of these have made news headlines.

The selective herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is a synthetic auxin that kills dicotyledonous (broadleaf) plants but has relatively little effect on grasses (incidentally an excellent example of plants differing in sensitivity to the same dose of hormone), and therefore found widespread use for removing dicotyledonous weeds from lawns and cereal crops. Its inclusion in the chemical warfare substance Agent Orange is notorious, but in fact it was an impurity (a highly carcinogenic dioxin compound) in the commercial preparation which led to worldwide withdrawal of the chemical. 2,4,5-T itself is not particularly toxic (Table 9.3). Preparations of a related compound, 2,4-D (2,4-dichlorophenoxyacetic acid) contain no dioxins and are still used as herbicides and in plant tissue culture.

Alar, also known as daminozide, is a plant growth retardant which was used widely on apples to modify fruit shape and skin colour. Some evidence in the 1980s suggested chronic toxicity symptoms were attributable to this chemical, and it was rapidly withdrawn from use. Sales of apples, even untreated ones, plummeted at the time, a dramatic example showing how a small number of scientific data can have massive economic consequences. Subsequent investigations concluded that there were no substantiated toxic effects but, harmful or not, the lasting impression in the general public has meant Alar has not been widely reintroduced (Caswell *et al.* 1991; O'Rourke 1990). This treatment was never essential for fruit production because it was used mainly for cosmetic changes: concerning size and appearance rather than to improve yield or nutritional quality. In general, legal restrictions, safety concerns and public perceptions are leading researchers and agrochemical companies to seek alternative means to achieve the same results, some of which are described next.

Table 9.3 Toxicity values for some plant growth regulators and other common organic chemicals. The LD_{50} test is a standard measure of acute toxicity, indicating the dose per kilogram of body weight required to kill 50% of a population. Clearly, the dioxin compound is vastly more toxic than any other on this list. However, using these data in isolation, DDT could be described as four times less harmful than caffeine, yet much of the human population deliberately ingests caffeine on a daily basis, but would be unlikely willingly to consume DDT. This illustrates how easily scientific data can be misconstrued. Long-term 'chronic' exposure may in fact be much more common with agricultural chemicals and may result in quite different toxicities, often with quite different relative hazards than the acute test, effective at much lower doses and much harder to attribute to the suspected chemical

Compound	LD_{50} (mg kg^{-1} orally in laboratory animals)
Plant growth regulators	
Alar (daminozide)	8400
Cycocel (CCC)	54
NAA (auxin)	1000
2,4-D (auxin)	370
2,4,5-T (auxin)	390
Dioxin (impurity in 2,4,5-T)	0.022
Other agrochemicals	
DDT (insecticide)	500
Aldrin (insecticide)	7
'Everyday' chemicals	
Thiamine (vitamin B)	8200
Caffeine	120
Nicotine	320
Paracetamol	338
Aspirin	1100

(Source of data: Merck Index)

9.3.2 Control though genetic alterations

A more stable and permanent way to alter plant development is through genetic modification. Essentially we depend on *mutations*, which can be spontaneous or induced by mutagens (DNA-altering chemicals or high-energy radiation such as X-rays, γ-rays or fast neutrons), and genetic engineering which allows us to remove, add or modify the expression of specific genes. The colossal expansion of *genetic engineering* since the mid-1980s is the single most remarkable change in biological research, and is covered further in Chapter 10. Here we look at some successes, pitfalls and limitations of plant genetic manipulation of hormonal signalling.

Genes for a few hormone biosynthesis enzymes have been isolated: these include one cytokinin and two auxin genes from plant pathogenic bacteria such as *Agrobacterium*, whose gall or tumour symptoms on infected plants relate specifically to the extra auxin and cytokinin produced — another example of the delicate hormone balance required for normal development. Plant genes for the two final steps of ethylene biosynthesis (ACC synthase and ACC oxidase; see p. 000) have been cloned, and there are now reports of isolation of ABA genes (Marin *et al.* 1996) and some of the many gibberellin biosynthesis genes (e.g. Phillips *et al.* 1995). There are impressive examples of applications of hormone biotechnology in retarding senescence of cut flowers (Figure 10.45) or controlling rates of ripening in stored fruit, but there are also significant gaps: we do not yet have isolated *plant* genes for auxin and cytokinin biosynthesis, or auxin- or cytokinin-deficient mutants, which hinders our interpretation of exactly which processes these hormones regulate. One view is that these two hormone classes are so essential to normal organised plants that deficiency would be a lethal character. Alternatively, multiple copies of biosynthetic genes may give plants a back-up system for continuing hormone production. In both cases, it is likely to be very difficult to screen for such mutations. A better target might be leaky mutations with only a partial restriction of hormone production. On the positive side, the array of gibberellin-related dwarfs clearly demonstrates non-lethal phenotypic alterations due to single gene mutations. Plants hampered in gibberellin, ABA or ethylene biosynthesis or perception are altered in specific developmental or physiological characters, but otherwise develop quite normally and most are reasonably fertile.

Achieving suitably precise control of transgene expression is difficult and severity of mutations is unpredictable, leading to many undesirable phenotypes. For example, a recurring problem has been from overexpression of the *IPT* gene, leading to high cytokinin levels, which in turn strongly inhibit root initiation and prevent recovery of whole plants from tissue culture. Remembering how tightly regulated plant developmental signals are, there is a pressing need to have suitable promoters, usually developmental stage specific, tissue specific or inducible by simple external factors such as O_2 concentration, copper ions or heatshocks, to drive gene expression in the right tissue, at the right time and to the right strength. Often, we have inadequate knowledge of hormone physiology to predict all these variables in advance, so research proceeds in a 'trial and error' fashion. Results often provide significant advances in basic understanding, even if the transgenic plants are not commercially useful.

The term 'billion dollar genes' has evocatively been given to genes that affect ripening and senescence, because this is a rough estimate of the value of the annual losses that occur worldwide due to fruit becoming overripe or too soft, or flowers wilting or foliage yellowing during the period from harvest to consumer. Most research has targeted high-value, perishable horticultural commodities rather than easier to handle grains and processed products. Since the 1980s, key achievements towards gaining genetic control of postharvest physiology include:

- isolation of the two plant genes necessary for ethylene biosynthesis;

- isolation of one bacterial gene for cytokinin biosynthesis;
- isolation of genes for enzymes catalysing pectin degradation, a major element of fruit cell wall softening;
- the ability not only to insert additional genes but to switch off effectively existing ones with methods such as 'antisense' or 'co-suppression' technology.

Commercial genetically engineered products have now been released, such as the FlavrSavr tomato, in which the polygalacturonase gene that normally leads to rapid fruit softening has been switched off by antisense RNA (Figure 11.22). Many more products of this type are in development. Tomato was selected as suitable for first trials because it is a major crop around the world and it is in the family Solanaceae which is generally amenable to biotechnology. There are also non-ripening tomatoes that have greatly reduced ethylene biosynthesis in the fruit. These can be ripened by exposure to ethylene gas, but not until they reach the market. Ethylene 'gassing' has been used for many years on normal tomatoes, as well as on bananas and citrus.

Carnations have been developed whose flowers do not show the normal rapid senescence, characterised by rolling up and wilting of the petals (Figure 10.45). These either have one of the ethylene biosynthesis enzymes blocked or have the bacterial cytokinin *IPT* gene inserted, which affects the normal balance of senescence regulation, where ethylene is promotive and cytokinin is inhibitory. Many other 'valuable' genes are being sought, such as those which might give a novel flower colour (Figure 10.45), or confer disease or pest resistance without the need for chemicals or lengthy conventional breeding programs. Some of these are discussed in Chapter 10.

Auxin and cytokinin genes: transformation to rooty and shooty phenotypes

Agrobacterium tumefaciens, the causative agent of crown gall disease, generates its symptoms and harnesses the plant's resources by inserting some of its own genes into the host DNA. This natural form of transformation has been exploited in many ways, and *Agrobacterium* remains one of the most popular means of inserting other genes into plants. Pathogenic transformation involves the Ti (tumour-inducing) plasmid, a circular piece of DNA containing the genes, two auxin and one cytokinin, necessary for biosynthesis of these two hormones. There are other plasmid genes associated with virulence and amino acid metabolism which we will not discuss here. Once the host is transformed, the bacteria are no longer required and symptoms persist due to the disruptive effect of excess auxin and cytokinin on plant cell development and organisation, very much akin to the callus seen in tissue culture or around a wound site on an intact plant. Other bacteria carry very similar genes, for example *A. rhizogenes*, *Pseudomonas savastanoi* and *Corynebacterium fascians* (Gaudin *et al.* 1994). Our notions of the respective roles of auxin and cytokinin in cell organisation are confirmed by experiments where one of the hormone genes has been deleted by mutation. The 'rooty' mutant phenotype is due to a non-functional cytokinin gene because preponderance of auxin is a root-inducing stimulus. Likewise 'shooty' tumours result from mutation of one or other of the auxin genes, because high cytokinin leads to shoot initiation.

Gibberellin dwarfs and dormancy

Dwarf mutant plants of pea, rice and maize have helped enormously in defining the role of gibberellins in stem elongation. Many widely used commercial cultivars such as dwarf pea or short straw wheat contain reduced quantities of active gibberellins, or have an inability to respond to gibberellin. From mutations at different loci and alleles of different 'strengths', it has been possible to establish the relationship between *endogenous* gibberellin content and growth in pea (Figure 9.14). This shows a remarkable similarity to the plots of *exogenous* gibberellin and growth. Internode lengths, the final expression of what was a growth rate, vary linearly with the log of gibberellin concentration. Gibberellin deficiency in *Arabidopsis* is quantitatively related to stem growth too, but also to seed dormancy and fruit setting: the more severely deficient genotypes require added gibberellin to stimulate normal germination, and then a continued supply of gibberellin to support stem elongation and fruit development. Interestingly, most gibberellin-deficient genotypes are unaffected in time or extent of floral initiation, suggesting either that gibberellins are not involved in flowering control, or that this function is restricted only to some species, or that there are specific gibberellins for flowering that are different from those involved in stem elongation and dormancy.

ABA mutants are wilty and have reduced seed dormancy

ABA also appears to have a role in seed dormancy, but one that is to some extent opposite to the gibberellin effect. Tomato, wheat, pea and *Arabidopsis* mutants deficient in ABA synthesis or ABA response exhibit minimal seed dormancy (Leonkloosterziel *et al.* 1996; Ooms *et al.* 1993). Interestingly, added ABA does not usually prolong dormancy, so the role of ABA is probably in the entry into dormancy during seed maturation rather than in exit from dormancy prior to germination. In addition, ABA mutants are very sensitive to water stress because they have poor control over their stomatal apertures, normally an ABA-mediated process. These 'wilty' plants have to be nursed in high-humidity chambers to prevent desiccation. Plants with poor drought tolerance are unlikely to be commercially valuable, but they are useful tools for testing the role of ABA in stress physiology. In the future, ABA genes may be manipulated to give *enhanced* response to or levels of ABA, thus improving stress resistance and water use efficiency, two valuable attributes for cultivated plants in many parts of the largely dry Australian continent.

9.3.3 Conclusions: the future of plant hormone research

For many years, plant hormone research focused on measurement of hormone levels. Based on responses to applied growth regulators, a widespread notion has been that plants regulate many developmental processes by actively modifying endogenous hormone concentrations. However, despite extremely sensitive and accurate assay techniques, there remain scant examples where normal plants (i.e. not mutant, not inhibitor treated, not genetically engineered) show large changes in endogenous hormone concentration *at the site of action*. Changes are usually much smaller than predicted, with some exceptions such as 50-fold increases in xylem ABA delivered to leaves in response to water status or 20-fold cytokinin level increases during dormancy release (Tardieu and Davies 1992; Turnbull and Hanke 1985). Much discussion through the 1980s permanently altered ideas on how hormones work in plants, in particular shifting the focus to control of hormone perception and sigual transduction, not just to the control of signal levels. The notion of control by changing tissue sensitivity to hormones is not new, but was vigorously proposed by Trewavas (1981) and others. However, in the absence of well-defined receptors, this theory was hard to test other than by traditional dose–response biological assays. Sensitivity is normally equated with presence of a suitable receptor system, but insensitivity (i.e. lack of response) can be the result of failure of any one of the many events between receptor and final physiological action, or sometimes the side effect of disruption of quite unrelated processes. The upshot has been expanded research on signal transduction (Trewavas and Malhó 1997), and a more balanced approach to the possible means of regulating hormone signalling and action.

As discussed above, attempting to modify plant development through applying PGRs has been popular for many years, often referred to as the 'spray and pray' or 'spray and weigh' approach. Since the late 1980s, by inserting genes for enzymes of hormone biosynthesis into plants or modifying their expression, alternatives to PGR treatments have been generated. In this way, a plant modifies its own hormone concentrations, avoiding the need for external applications and potentially reducing amounts of chemicals used in agricultural and food industries. However, the responses are very similar in both cases: often in addition to the desired response, we find one or more side effects which frequently limit the usefulness of either technique. The problem lies not only in the multiple functions of plant hormones but also in their mobility within the plant; for example, it is quite hard to prevent root-synthesised cytokinin moving to the shoot in the xylem sap. We conclude this chapter with an idea: if instead of genetically altering hormone *concentrations*, the receptor or downstream events are modified, we may be then able to generate much more precise tissue-specific control. Receptors, being proteins, will be effectively immobile. Indeed, we already know that some hormone receptor genes are regulated naturally during development. In tomato fruit, the *tETR* gene which codes for an ethylene-binding protein is hardly expressed at all until the fruit starts to ripen (Figure 9.15). This means that the fruit remains very insensitive to ethylene until the stage of development when the seeds are mature. Regulation of a hormone response in this case confers an adaptive advantage by reducing the likelihood of premature triggering of ripening and seed dispersal before maturity. Much current research is seeking ontogenetically regulated and especially tissue-specific promoters to link to a wide range of genes including those responsible for regulation of hormone concentrations, and this could now be extended to include genes for hormone perception and action.

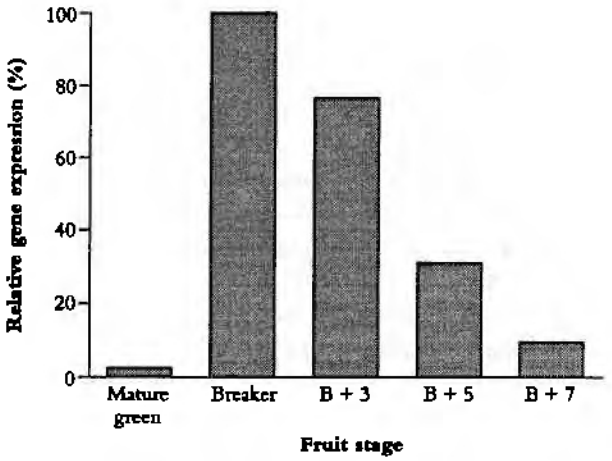


Fig 9.15 Although ripening of climacteric fruit such as tomato is normally associated with autocatalytic ethylene synthesis (see Chapter 11), the physiological response to this increased ethylene level depends on presence of ethylene receptors. In this experiment, the expression of the *tETR* gene, which codes for a presumed receptor, was quantified by the relative amount of *tETR* mRNA present. In mature green fruit, the gene is scarcely expressed, but maximal levels are present during the first visible stages of ripening colour change known as 'breaker' (B). Fruit are fully red by seven days after breaker (B + 7), by which time receptor gene expression has declined again. The developmental regulation of receptor levels may be a key safeguard preventing premature onset of ripening until the fruit and seed are at the right stage of development. Deliberate manipulation of hormone receptor expression by genetic engineering may become a powerful tool for controlling tissue-specific and developmental-time-based hormone responses
(Redrawn, with permission, from Payton *et al.* 1996)

Further reading

Abeles, F.B., Morgan, P.W. and Saltveit, M.E. (1992). *Ethylene in Plant Biology*, 2nd edn, Academic Press: New York.

Davies, W.J. and Jones, H.G. (eds) (1991). *Abscisic Acid: Physiology and Biochemistry*, Bios Scientific: Oxford.

Evans, P.T. and Malmberg, R.L. (1989). 'Do polyamines have roles in plant development?', *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 235–269.

Fry, S.C., Aldington, S., Hetherington, P.R. and Aitken, J. (1993). 'Oligosaccharides as signals and substrates in the plant cell wall', *Plant Physiology*, **103**, 1–5.

Hildman, T., Ebneth, M., Pena-Cortes, H., Sanchez-Serrano, J.J., Willmitzer, L. and Prat, S. (1992). 'General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding', *Plant Cell*, **4**, 1157–1170.

Lazarus, C.M. (1991). 'Hormonal regulation of plant gene expressio', in *Developmental Regulation of Plant Gene Expression*, ed. D. Grierson, 42–74, Blackie: Glasgow.

Malamy, J. and Klessig, D.F. (1992). 'Salicylic acid and plant disease resistance', *Plant Journal*, **2**, 643–654.

Mohr, H. and Schopfer, P. (1995). *Plant Physiology*, Springer-Verlag: Berlin.

Parthier, B. (1991). 'Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions', *Botanica Acta*, **104**, 405–464.

Reid, J.B. (1990). 'Phytohormone mutants in plant research', *Journal of Plant Growth Regulation*, **9**, 97–111.

Sasse, J.M. (1997). 'Recent progress in brassinosteroid research', *Physiologia Plantarum*, **100**, 696–701.

Verhey, S.D. and Lomax, T.L. (1993). 'Signal transduction in vascular plants', *Journal of Plant Growth Regulation*, **12**, 179–195.

Vleeshouwers, L.M., Bouwmeester, H.J. and Karssen, C.M. (1995). 'Redefining seed dormancy — an attempt to integrate physiology and ecology', *Journal of Ecology*, **83**, 1031–1037.

Zeevaart, J.A.D. and Creelman, R.A. (1997). 'Metabolism and physiology of abscisic acid', *Annual Review of Plant Physiology and Plant Molecular Biology*, **39**, 439–473.